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TABLE OF CONTENTS FIRST PAGE

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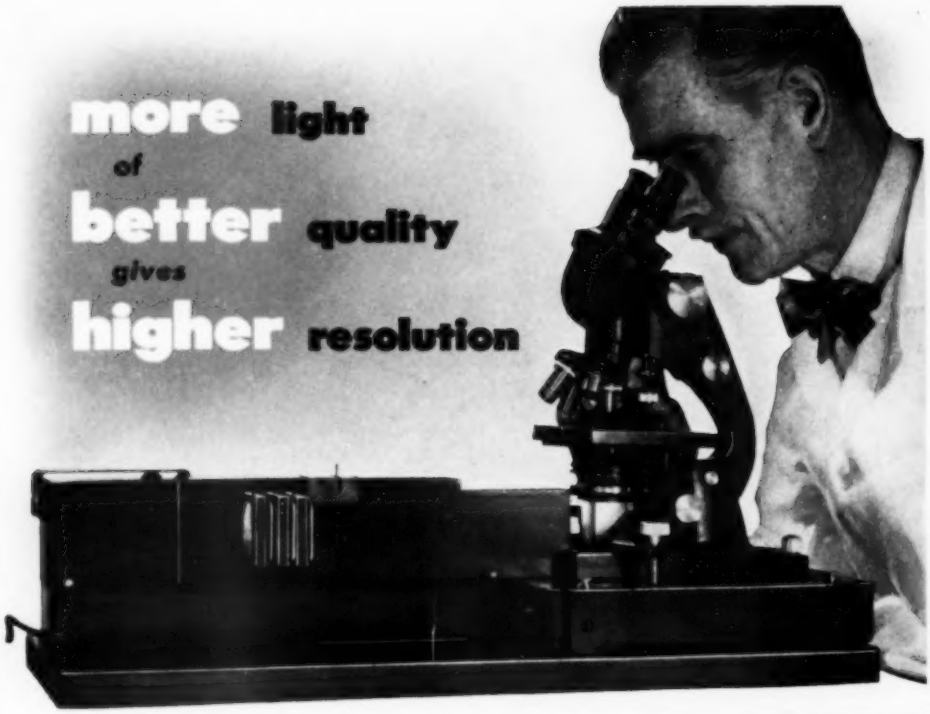

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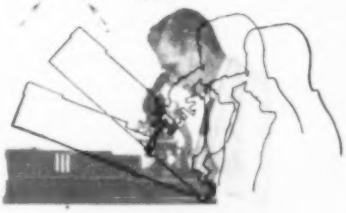

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# CONTENTS

## Original Articles


**Stomach Size and Its Relation to Chronic Peptic Ulcer** PAGE
*Alvin J. Cox Jr., M.D., San Francisco* ..... 407

**Osteoarthropathy in Dogs**
*Hugo Stunzi, Boston* ..... 423

**Pulmonary Reaction to Barium Sulfate in Rats**
*John Huston Jr., M.D.; D. P. Wallach, and George J. Cunningham, M.D., Madison, Wis.* ..... 430

**Observations on Specificity of Schultz Histochemical Test for Demonstrating Cholesterol in Tissue**
*Sidney P. Kent, M.D., Birmingham, Ala.* ..... 439

**Effect of Severe Acute Hemorrhage on Kidney of Rat**
*Melvin A. Block, M.D.; Khalil G. Wakim, M.D., and Frank C. Mann, M.D., Rochester, Minn.* ..... 443

**Ribonucleic Acid in Cytoplasm of Liver Cells**
*W. J. Pirozynski, M.D., and L. von Bertalanffy, Ph.D., Ottawa, Ont., Canada* ..... 450


## Case Reports


**Dissecting Intramural Hemorrhage of Anterior Descending Branch of Left Coronary Artery**
*William V. Lovitt Jr., M.D., and W. J. Corzine Jr., M.D., Baltimore* ..... 458


## General Review


**Diffuse Glomerular Nephritis and Lipid Nephrosis**
*William E. Ehrich, M.D.; Carolyn W. Forman, and Joseph Seifer, Philadelphia* ..... 463


## Regular Departments


**News and Comment** ..... 504

**Books** ..... 505

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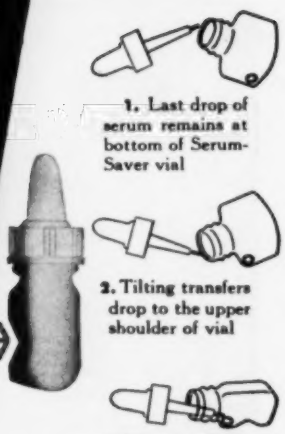

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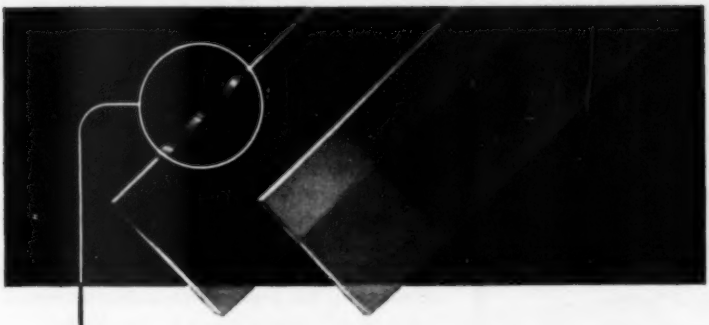

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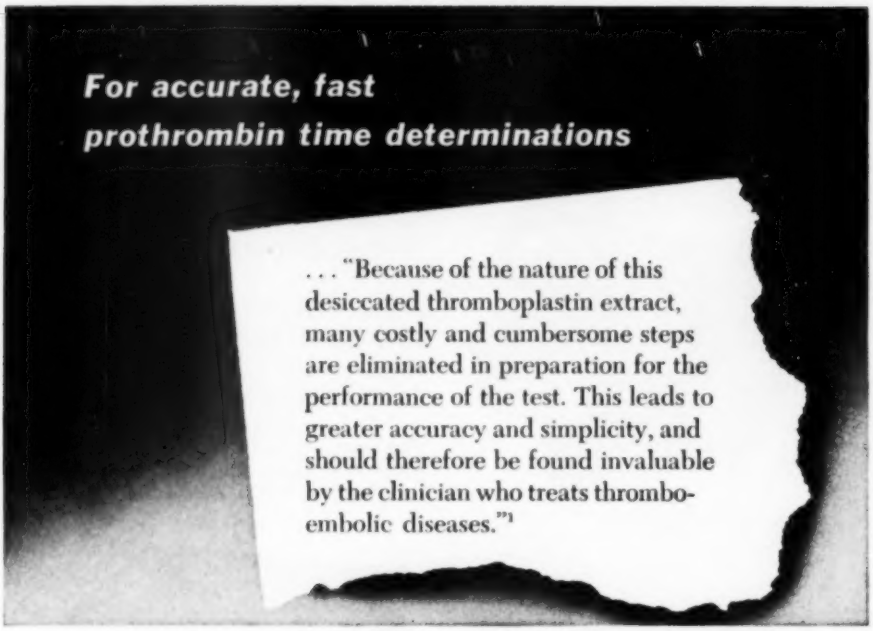
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1. Am. J. M. Sc. 222:207 (Aug.) 1951.
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# STOMACH SIZE AND ITS RELATION TO CHRONIC PEPTIC ULCER

ALVIN J. COX Jr., M.D.
SAN FRANCISCO

THE TERMS "atrophic" and "hypertrophic" have been used frequently with respect to the stomach, particularly in the classification of chronic gastritis, but there have been few objective measurements of stomach size, and little attention has been given to variations in the measured quantity of mucosal tissue in this organ. Clinical methods have not supplied accurate measurements, and satisfactory quantitative information is difficult to obtain even from anatomical specimens. Freeman [1] reported that 76% of his cases of duodenal ulcer presented a hyperplastic type of gastric mucosa characterized gastroscopically by thick crowded mucosal folds. Most other gastroscopists have not described such a consistent relationship, although a number of reports suggest an unusual frequency of chronic hypertrophic gastritis in patients suffering from duodenal ulcer. Wangensteen and his associates [2] noted the impression that duodenal ulcers are more likely to be accompanied by large stomachs than are gastric ulcers; they pointed out that duodenal ulcers more often cause obstruction (a possible cause of gastric enlargement), and that gastric ulcers often result in scarring and contraction of the stomach. Hebbel [3] found that in 14 of 78 stomachs removed at operation from patients with duodenal ulcer, and in 2 of 13 cases of associated duodenal and gastric ulcers, the mucosa was more than 1.5 mm. thick in the body portion, whereas none of his cases of gastric ulcer alone or of his autopsy control series showed such a thick mucosa. Cox [4] mentioned briefly that the average gastric mucosal area in 17 cases of chronic or healed duodenal ulcer was greater than that in patients without ulcer or with gastric ulcer. An extension of the latter study has provided the material for this report.

## MATERIAL AND METHODS

Two hundred twenty-two human stomachs obtained at autopsy from patients of both sexes ranging in age from 17 to 85 years included 52 specimens from patients with chronic or healed gastric or duodenal ulcer. A wide variety of other diseases

From the Department of Pathology, Stanford University, School of Medicine.

1. Freeman, H.: Duodenal Ulceration: A Gastroscopic Study of the Gastric Mucosa and Its Surgical Significance, Brit. M. J. **1**:980, 1946.

2. Wangensteen, O. H.; Barco, R. L.; Hay, L.; Walpole, S., and Trach, B.: Gastric Acidity Before and After Operative Procedure, with Special Reference to the Role of the Pylorus and Antrum: Preliminary Report of Clinical and Experimental Study, Ann. Surg. **112**:626, 1940.

3. Hebbel, R.: Chronic Gastritis: Its Relation to Gastric and Duodenal Ulcer and to Gastric Carcinoma, Am. J. Path. **19**:43, 1943.

4. Cox, A. J.: Variations in Size of the Human Stomach, California & West. Med. **63**:267, 1945.

had been present; most had no gastric manifestations. A number of stomachs presented single or multiple tiny superficial acute ulcers, but these stomachs were distinctive in no other way. The presence of proliferated fibrous tissue at the ulcer base was required for identification of an ulcer as chronic. The stomach weight and mucosal area were measured in all specimens, and the average mucosal thickness, mucosal volume, and total number of parietal cells were estimated in a selected group.

The stomachs were removed within 12 hours, and in most cases no longer than 6 hours, after death. No specimen was included in which postmortem digestion had denuded the mucosa at any point. All were opened along the greater curvature and before fixation were stretched on a board just enough to flatten the mucosal folds completely. This degree of stretching was achieved with good uniformity and it permitted the measurement of mucosal area under uniform conditions. The gastric mucosa does not change its area greatly with different degrees of expansion of the stomach [5]; when a stomach is collapsed the lining is thrown into folds, and within the ordinary range of variation of stomach content the mucosal area can be regarded as nearly constant.

After fixation in the stretched position by immersion in 4% formaldehyde solution, an outline map was made by tracing the borders of each specimen on paper, and the mucosal area was measured with a planimeter. The specimens were weighed after fixation following removal of the attached peritoneal ligaments and masses of fatty tissue.

Three longitudinal mucosal strips were cut from each stomach along the entire length of the anterior wall, the posterior wall, and the lesser curvature, respectively. The strips were separated from the muscularis and were rolled up so all parts could be sectioned at once in a plane perpendicular to the mucosal surface. Histological sections cut in this way from the paraffin-embedded tissue were mounted carefully to avoid excessive separation of the tissue elements. The sites of removal of the strips were plotted upon the maps, and the location of the junction between the pyloric zone (antrum) and the fundic zone (body) was drawn in from corrected measurements made upon the histological sections (Fig. 1). This permitted measurements from the maps of the approximate area of each of the mucosal zones in each stomach, including the lesser curvature of the fundic zone, which was measured separately because its character did not always correspond to that of the remainder of the fundic zone. It was usually thinner and well demarcated at approximately the lines joining the borders of the opened esophagus with those of the opened pylorus. Accordingly, these lines have been used for the arbitrary delineation of the lesser curvature in all specimens.

Measurements of average mucosal thickness were made from the histological sections of 135 specimens in which there was only minimal postmortem change. No specimen was included in which the position of the mucosal surface could not be clearly identified. Images of the sections representing the pyloric and the fundic zones were projected onto paper with a magnification of 10 diameters, and from

5. Schmidt, A.: Zur Pathogenese des Magengeschwürs, Proceedings of the Twentieth Congress of Internal Medicine, Wiesbaden, 1902, p. 270. Bloch, C. E.: Beitrag zur Pathogenese des chronischen Magengeschwürs, Deutsche med. Wchnschr. **31**:1224, and 1272, 1905. Cole, L. G.: Hypertrophic Gastritis, M. Clin. North America **17**:1, 1933.

tracings, with the aid of a planimeter, the average thickness of the mucosa in each zone was calculated. Expressions of average thickness were necessary because there was much difference in thickness at different levels, with gradual diminution toward the fundus, as has been reported by Berger [6] and others. Since the thickness of the lesser curvature in the fundic zone was often quite different from that of adjacent regions, this area was considered separately. The average thickness in the remaining major portion of the fundic zone was derived from measurements of the two mucosal strips removed from the anterior and the posterior walls, respectively. This procedure was considered acceptable because of the comparable thickness of the mucosa at a given level in all parts of the circumference, with the exception of the lesser curvature, and because the circumference of the fundic zone was nearly uniform.

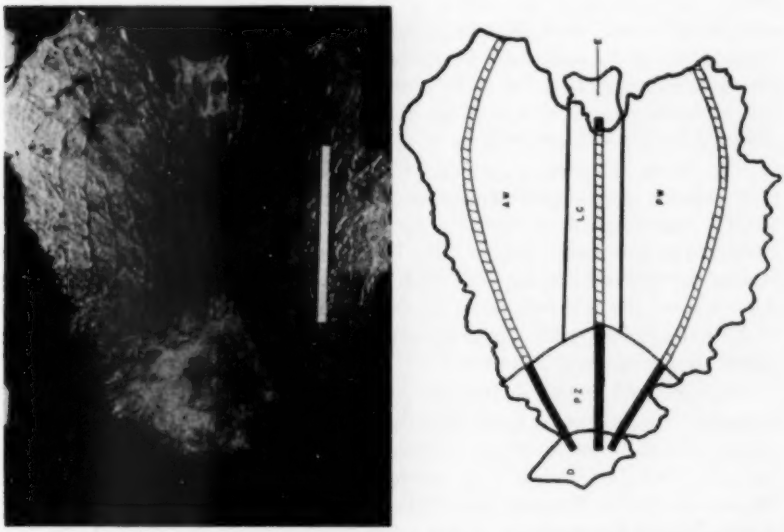


Fig. 1.—Stretched gross specimen of stomach prior to fixation, with corresponding map. The position of the mucosal strips removed for histological study, and the location of the junction between pyloric and fundic zones are indicated. The borders of the lesser curvature in the fundic zone were determined arbitrarily by drawing lines between the edges of the opened esophagus and the borders of the opened pylorus. *E*, esophagus; *D*, duodenum; *AW* and *PW*, anterior and posterior walls, respectively; *LC*, lesser curvature.

Sample measurements have shown less than 15% difference in average thickness measured in different mucosal strips along different longitudinal paths on the same anterior or posterior wall. The average mucosal thickness of the fundic zone, exclusive of the lesser curvature, provided a satisfactory expression for purposes of comparison, as the latter region rarely represented more than 15% of the total fundic zone area, and, though thinner than the remainder, it usually showed thickness variations comparable to those elsewhere within the zone. There was little

6. Berger, E. H.: Distribution of Parietal Cells: Histotopographic Study, Am. J. Anat. **54**:87, 1934.

difficulty in estimating the average mucosal thickness of the pyloric zone, as its thickness was fairly uniform.

In two of the stomachs there was no clear distinction between the pyloric zone and the fundic zone, because of the extensive abnormality of the latter; hence no separate measurements of these zones could be made. In six other specimens a similar lack of delineation of the zones was found on the lesser curvature, though not elsewhere.

Indices of mucosal volume were obtained by multiplying the measured areas by the average mucosal thickness in the corresponding zones. The fundic zone was treated as three parts—the anterior wall, the posterior wall, and the lesser curvature—while the volume index of the pyloric zone was calculated for the zone as a whole. These values do not express actual mucosal volume because the thickness measurements were made from histological sections of dehydrated tissue. The same errors affecting the estimation of average mucosal thickness have influenced the mucosal volume indices, but it is believed that the striking differences in these indices represent real variants. They corresponded to differences which could be observed without measurement.

The 135 stomachs which were used for measurements of mucosal thickness were also subjected to quantitative estimation of the number of parietal cells. In no case did the appearance suggest that a significant number of these cells had been destroyed by postmortem change. They lie deep to the mucosal surface and are not reached by mild surface digestion. They can be distinctively stained, and the fact that these are the cells responsible for acid secretion made it particularly desirable to determine their number. No attempt has been made to count other mucosal cells, which are distinguished less readily.

The cells were counted in a manner which has been reported in a study of animal stomachs.[7] In each of 36 representative bands 0.23 mm. broad, crossing perpendicularly to the mucosal surface of histological sections, all parietal cells in which the nuclei could be discerned were counted. From the thickness of the sections and the average nuclear diameter, which was not found to differ greatly relative to the number of cells, the number of nuclear points represented by each counted band was calculated by the method of Abercrombie.[8] The amount of mucosal surface represented by each counted band was calculated, and the total number of parietal cells in the fundic zone of each stomach was estimated. Corrections were made to compensate for the shrinkage of the mucosal strips during preparation of the histological sections. When making the counts the observer did not know the stomach areas or which cases exhibited peptic ulcer. Some inaccuracy is due to the use of single strips to represent zones, the sides of which were not exactly parallel, but it seems certain that inaccuracy of estimation of the number of parietal cells could not be responsible for the wide differences which were observed.

7. Cox, A. J., and Barnes, V. R.: Experimental Hyperplasia of the Stomach Mucosa, Proc. Soc. Exper. Biol. & Med. **60**:118, 1945.

8. Abercrombie, M.: Estimation of Nuclear Population from Microtome Sections, Anat. Rec. **94**:239, 1946.

## RESULTS

*Size Differences.*—The mucosal area of the 222 stomachs ranged from 421 to 1,536 sq. cm. The average values were 850 sq. cm. for males and 783 sq. cm. for females. There was a roughly parallel range in weight. The heaviest weighed nearly six times that of the lightest, with average values of 162 and 112 gm. for males and females, respectively. Much of the weight represented the submucosa and the muscularis, and the direct relationship to mucosal area demonstrates that differences in the latter were not simply the result of differences in the degree of stretching of the specimens. No consistent relationship has been observed between stomach size and age or body size. The size of the stomach has not reflected the presence of any of a wide variety of diseases in other organs.

TABLE 1.—*Relationship Between Stomach Mucosal Area and Average Thickness of the Mucosa in Histological Sections*

| Stomach Area (Sq. Cm.) | Number of Cases | Average Thickness (Mm.) * | |
|---|---|---|---|
| | | Fundic Zone (Body) | Pyloric Zone (Antrum) |
| 400-699 | 31 | 0.43 | 0.55 |
| 700-799 | 37 | 0.50 | 0.53 |
| 800-899 | 35 | 0.55 | 0.54 |
| 900-999 | 18 | 0.61 | 0.58 |
| 1,000 and over | 14 | 0.68 | 0.58 |

* Measurements from the anterior and posterior walls; the lesser curvature is not included.

TABLE 2.—*Relationship Between Stomach Mucosal Area and Volume of the Mucosa*

| Stomach Area (Sq. Cm.) | Number of Cases * | Average Index of Mucosal Volume (Cc.)† | | |
|---|---|---|---|---|
| | | Total | Fundic Zone (Body) | Pyloric Zone (Antrum) |
| 400-699 | 30 | 31 | 26 | 5 |
| 700-799 | 34 | 39 | 34 | 5 |
| 800-899 | 36 | 44 | 38 | 6 |
| 900-999 | 16 | 55 | 48 | 7 |
| 1,000 and over | 15 | 72 | 65 | 7 |

* Several cases were excluded because the junction between the mucosal zones was poorly defined.
† Calculated from thickness of histological sections.

Table 1 shows that the measured mucosal area was directly related to the average mucosal thickness, particularly that of the fundic zone mucosa, which usually represents 80 to 90% of the total mucosal area. In the small stomachs the average thickness of the fundic zone was usually less than that of the pyloric zone, while in the large stomachs it was greater. In 12% of the cases the difference in thickness of the two zones was more than 50% of the smaller value. When pronounced, this difference was easily seen in the gross specimen, as the transition was usually abrupt.

When the quantity of mucosal tissue was expressed in units of mucosal volume (Table 2), it was evident that most of the difference in stomachs of different sizes was the result of variations in the fundic zone mucosa, which is the specific secretory portion of the stomach. The pyloric zone contributed very little to the differences in total amount of gastric mucosa. The data also emphasize the fact that differences

in total mucosal area of the groups of stomachs reflected even greater differences in volume of the specific secretory tissue.

The shape of the large stomachs was somewhat different from that of the smaller specimens (Table 3). Most of the large preparations were relatively broad. The maximum circumference in the largest and smallest groups differed by 43%, while the corresponding measurements of length differed by only 17 and 21%. The length, therefore, contributed less to the increased area of the large stomachs than did the circumference, which frequently exceeded the length in the large specimens. This difference in shape relative to stomach size is consistent with the observation of exceedingly prominent longitudinal mucosal folds in the large stomachs. No quantitative measurements of the prominence of mucosal folds have been made, but in specimens which were not distended a roughly parallel relationship to mucosal area was observed. Folds were often inconspicuous or absent in the small stomachs.

In many of the large stomachs of this series the surface of the mucosa of the fundic zone was strikingly nodular. This produced a pebbly or cobblestone appearance suggesting the appearance frequently described in so-called chronic hypertrophic gastritis and resembling the illustration by Braus[9] which has been

TABLE 3.—*Dimensions of Stretched Specimens of Stomach in Relation to Mucosal Area**

| Stomach Area (Sq. Cm.) | Number of Cases | Lesser Curvature Length (Cm.) | Pylorus to Fundus (Cm.) | Maximum Circumference (Cm.) |
|---|---|---|---|---|
| 400-699 | 48 | 23.6 | 30.9 | 26.8 |
| 700-799 | 54 | 24.7 | 32.9 | 30.6 |
| 800-899 | 49 | 26.0 | 34.8 | 32.0 |
| 900-999 | 35 | 26.7 | 35.6 | 34.0 |
| 1,000 and over | 28 | 27.5 | 37.3 | 38.3 |

* Specimens with gastric scars were omitted.

reproduced in many textbooks of histology as a representation of normal gastric mucosa. The surface irregularity was most developed over the thickest parts of the mucosa, usually away from the lesser curvature in the distal portion of the fundic zone. The nodular protuberances often had the form of anastomosing ridges separated by linear depressions (Fig. 2). When pronounced, the nodular character of the surface was readily visible before fixation; minor irregularities were more easily identified after fixation in formaldehyde solution. Comparable surface irregularity was absent from the pyloric zone, although in some cases the surface here was irregular or finely granular in a less distinctive fashion.

Table 4 illustrates a relationship between mucosal thickness and the presence of a nodular mucosal surface in the fundic zone.

A thin mucosa was never markedly nodular, whereas all the very thick specimens presented prominent surface nodularity. All but one of the stomachs with marked mucosal nodularity were larger than the average; the one exception had a mucosal area of 753 sq. cm.

When the surface was nodular, the microscopical appearance of the mucosal glands was similar in the thick and the thin portions, although the glands beneath the furrows were short (Fig. 3). No evidence has been found to indicate that these depressions resulted directly from any pathological process.

9. Braus, H.: Anatomie des Menschen, Berlin, 1924, Vol. 2, p. 230.

*Measurements in Cases of Peptic Ulcer.*—In Figure 4, gastric mucosal area is related to age, and it can be seen that age is not a factor of importance in determining mucosal area. In 30 cases of chronic duodenal ulcer or scar the average mucosal area was 938 sq. cm. (946 sq. cm. in 24 males and 907 sq. cm. in 6 females).

TABLE 4.—*Relationship of Nodular Irregularity* of the Fundic Zone Mucosal Surface to Average Mucosal Thickness in Histological Sections*

| Thickness of Fundic Zone (Mm.) † | Number of Cases | Degree of Surface Irregularity † (Per Cent of Cases) | | |
|---|---|---|---|---|
| | | None or Slight | Moderate | Marked |
| <0.5 | 51 | 92 | 8 | 0 |
| 0.5-0.8 | 76 | 43 | 44 | 12 |
| >0.8 | 8 | 0 | 0 | 100 |

* Estimates were made after formalin-fixation of the specimens.
† Measurements from the anterior and posterior walls.

Fig. 2.—Photograph before fixation of portion of stretched mucosal surface from fundic zone of large stomach with thick mucosa, showing surface nodularity. The white streaks represent highlights.

This contrasts with corresponding values of 825 and 782 sq. cm. for males and females without ulcer or scar. Only four (13%) of the stomachs from the duodenal ulcer patients fell below the average stomach size for the control group of corresponding sex, and the smallest stomach of the ulcer group was only 10% below this control value.

In the 13 cases in which there was duodenal scarring but no open ulcer the average stomach mucosal area was 905 sq. cm., while the stomachs in the 17 cases with active duodenal ulcer averaged 964 sq. cm. in mucosal area. This difference is not statistically significant. There was no recognizable size difference related to whether the ulcers were single or multiple. No peculiarities of the large stomachs associated with duodenal ulcer distinguished them from other large stomachs.

A number of the 22 stomachs bearing chronic gastric ulcers or scars, and not accompanied by duodenal lesions, were unusually small. Seventeen were smaller than the control average. The stomachs from 13 males of this group averaged 778 sq. cm.; the average for 9 females was 653 sq. cm. The small size of these organs was due at least in part to contraction of zones of fibrosis, which were sometimes

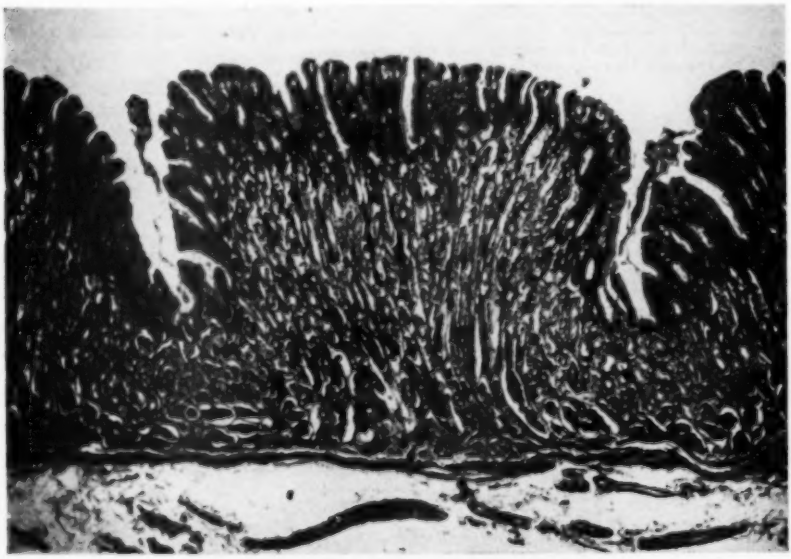

Fig. 3.—Photomicrograph of gastric mucosa from a thick nodular fundic zone. The mucosal glands in the thick and thin portions are similar. There is insignificant gastritis.

conspicuous. The 13 stomachs with open ulcers (8 from males and 5 from females) averaged 744 sq. cm., and the average for the 9 stomachs which presented only scars (5 from males and 4 from females) was 619 sq. cm. This difference, which is not highly significant statistically, may represent the result of more fibrous tissue contraction around healed ulcers than in the vicinity of open ulcers. Several examples demonstrated that gastric ulcers sometimes developed in large stomachs, and it seems apparent that, although the average size of the stomachs bearing gastric ulcers was distinctly smaller than that of the controls, this was probably not of fundamental significance.

In two additional cases duodenal scars were present in addition to gastric ulcers. In these cases the gastric mucosal areas were 923 and 1,024 sq. cm. respectively. These stomachs corresponded in size to those of the duodenal ulcer group.

*Histological Studies.*—Since most of the substance of the gastric mucosa is made up of mucosal glands except in infrequent instances of severe disease, one might expect a relationship to exist between the amount of mucosa in the fundic zone and the secretory capacity of the stomach. Table 5 indicates that there was a relationship between the number of parietal cells and the quantity of fundic zone mucosa in a group of 127 stomachs. The volume of mucosa did not reflect the number of parietal cells accurately, however, as there was considerable variation of the latter, dependent

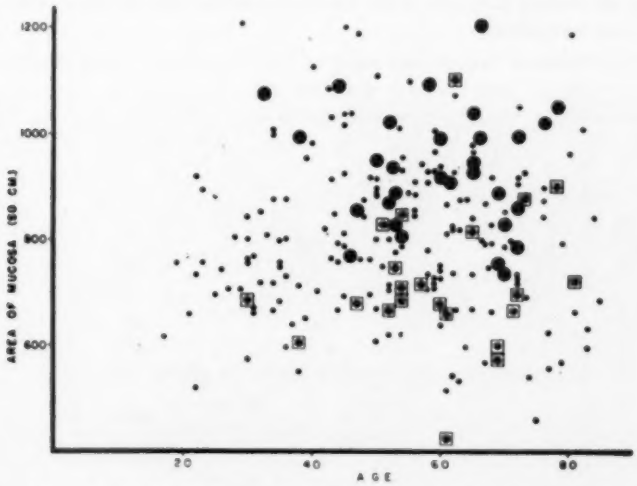


Fig. 4.—Mucosal area of 221 stomachs. Large solid circles represent cases of chronic duodenal ulcer or scar. Squares represent cases of chronic gastric ulcer or scar. One case with a mucosal area of 1,536 sq. cm. but no ulcer is not included.

TABLE 5.—*Volume of Fundic Zone Mucosa and Estimated Number of Parietal Cells*

| Index of Mucosal Volume (Ml.) | Number of Cases | Parietal Cells per Stomach (Average in Billions) | Distribution of Cases (Per Cent) | | |
|---|---|---|---|---|---|
| | | | 1 Billion | 1-2 Billion | 2 Billion |
| 10-30 | 41 | 0.6 | 81 | 19 | 0 |
| 31-50 | 65 | 1.2 | 35 | 59 | 6 |
| Above 50 | 21 | 1.9 | 5 | 47.5 | 47.5 |

upon different degrees of separation of the mucosal glands by infiltrated lymphocytes and plasma cells, and resulting also from differences in the number of parietal cells per mucosal gland. There were pronounced differences in the proportion of the glands formed by the mucus-secreting cervical portion, which is almost devoid of parietal cells. In several stomachs this portion constituted as much as half the length of the glands, and when there was accompanying infiltration of lymphoid cells the picture was that which Schindler and Ortmayer [10] have termed chronic

10. Schindler, R., and Ortmayer, M.: Histopathology of Chronic Gastritis, Am. J. Digest. Dis. **9**:411, 1942.

proliferative hypertrophic gastritis. In other cases the cervical portion of the glands was less than 10% of the total length.

In Figure 5 the estimated number of parietal cells in each of the 135 stomachs is plotted against the age of the corresponding patients. Age had little influence upon the occurrence of large numbers of parietal cells, but stomachs containing few parietal cells belonged more commonly to old persons. This was related to the greater incidence of chronic gastritis in old people. The stomachs from males contained more parietal cells than were found in females; the averages were 1.18 and 0.84 billion, respectively.

Chronic duodenal ulcer or scar occurred only in association with stomachs which contained relatively large numbers of parietal cells. In each of the 15 duodenal ulcer cases in which the stomach was suitable for parietal cell estimation there were more

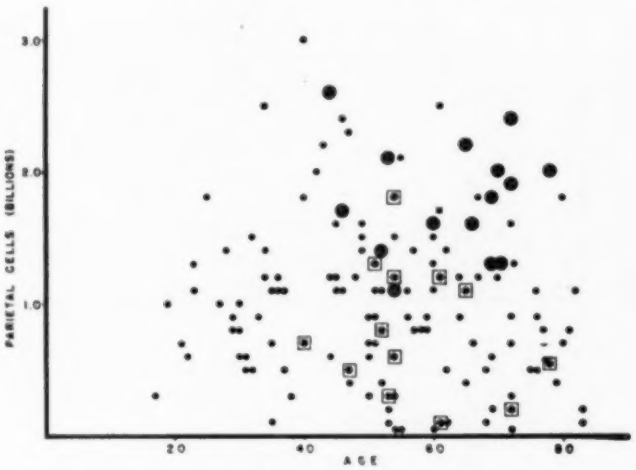


Fig. 5.—Estimated total number of parietal cells in 135 stomachs. Large solid circles represent cases of chronic duodenal ulcer or scar. Squares represent cases of chronic gastric ulcer or scar.

such cells than the average for the corresponding sex. The average parietal cell value for all the duodenal ulcer cases was 1.8 billion (1.9 billion for 11 males and 1.52 billion for 4 females). The incidence of duodenal ulcer or scar was distinctly higher in the stomachs of patients aged 60 or older in which there were larger numbers of parietal cells than in similar stomachs from young persons. There was no clear difference in the number of parietal cells in seven instances of duodenal scar and eight cases of active duodenal ulcer.

In 13 stomachs of widely differing parietal cell content there were gastric ulcers or scars. Several contained 0.3 billion or fewer parietal cells. The average parietal cell value was 0.8 billion. In one case in which gastric ulcer and duodenal scar coexisted the parietal cell value was 1.6 billion, which is comparable to that in the other cases of duodenal ulcer.

*Relation of Mucosal Size to Chronic Gastritis.*—Chronic gastritis has been variously defined. It is generally accepted that pronounced infiltration of the gastric mucosa by lymphoid cells or qualitative alteration of mucosal glands represents disease, but since minor degrees of the same anatomical changes are extremely common, their significance has been questioned. The term "physiological gastritis" has been used.[11] Little information is available concerning the relation of gastritis to stomach size. In the series of cases studied here there was no clear relationship between qualitative changes in the pyloric zone and stomach size. The amount of cellular infiltration in the fundic zone was not appreciably related to stomach mucosal area in most cases, but severe changes in the mucosal glandular pattern, with loss of most or all of the parietal cells, reflected the presence of a small stomach

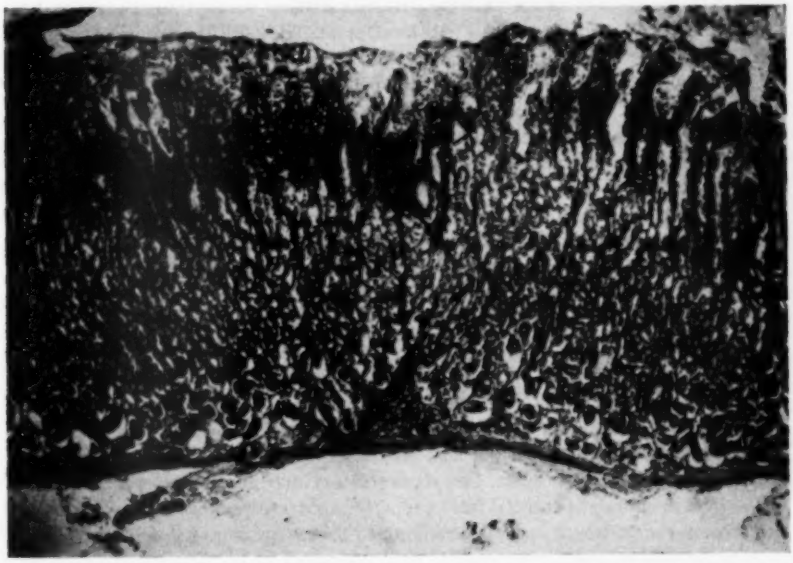

Fig. 6.—Photomicrograph of fundic zone mucosa in which lymphocytes and plasma cells are concentrated in the superficial portion, a region very poor in parietal cells.

in 22 of 24 cases, 5 of which were instances of known pernicious anemia. In this group the average mucosal area for 15 males was 702 sq. cm., and for 9 females was 680 sq. cm.

Eighty-five (63%) of the stomachs in which the number of parietal cells was estimated exhibited few or no lymphoid cells in the fundic zone mucosa. In about half these cases the infiltrated cells were closely placed but were limited largely to the superficial portion of the mucosa where there were but few parietal cells (Fig. 6). These 85 cases of mild cellular infiltration of the fundic zone mucosa correspond

11. (*a*) Hamperl, H.: Zur Histologie der akuten Gastritis und der Erosionen der Magenschleimhaut: Ergebnisse der deutsch-russischen Rassenforschung, Beitr. path. Anat. **90**:85, 1932. (*b*) Benedict, E. B., and Mallory, T. B.: Correlation of Gastroscopic and Pathological Findings in Gastritis, Surg., Gynec. & Obst. **76**:129, 1943.

to Grades I and II of Benedict and Mallory,[11b] who regarded the infiltration as normal. It is doubtful that any significant reduction in the number of parietal cells resulted from the presence of cellular infiltration of this degree. Even in the almost complete absence of lymphocytes and plasma cells from the mucosa, there were wide differences in the number of parietal cells in different stomachs, indicating that factors other than gastritis determine the number of parietal cells, and it is apparent that the development of a thick fundic zone mucosa in these cases was not dependent upon gastritis. Although the term hyperplasia might properly be used in naming these changes, "hypertrophic gastritis" is not applicable.

*Symptoms Associated with Large Stomachs.*—No comprehensive study has been made of the occurrence of clinical symptoms in the cases reported here, but a survey of the hospital records of the patients with stomachs of mucosal area greater than 1,000 sq. cm. has been carried out. All these stomachs had a large mucosal volume and many parietal cells. Those patients with active or healed duodenal ulcer frequently had symptoms referable to the stomach, but of 20 patients without ulcer there were only 2 with abdominal symptoms which could not be easily explained by conditions such as liver cirrhosis, uremia, and malignant neoplasm. One of the two exceptions was a patient with coronary arteriosclerosis who had had episodes of nausea, vomiting, and abdominal pain for six years. The other patient, suffering from chronic alcoholism, had repeated symptoms referred to the stomach. Two records were inadequate to supply any information regarding abdominal symptoms. In five instances the patients had specifically denied gastrointestinal symptoms of any kind. This limited survey suggested that the presence of a large stomach per se was not the cause of clinical symptoms.

## COMMENT

The modification of mucosal structure by agonal and postmortem changes has been the basis for criticism of observations made on specimens of stomach removed post mortem.[12] There is no doubt that postmortem changes occur regularly in the gastric mucosa soon after death, and if severe they may render exact observations useless; yet much can be learned from properly prepared specimens. One must take into account the postmortem changes but not to the extent of giving up observations.

It is possible to reduce the amount of postmortem digestion of the stomach by injection of dilute formalin or other fixative into the cavity of the organ immediately after death, but this prevents satisfactory stretching of the mucosa when it is removed later, and the folded mucosa is difficult to study quantitatively. The area cannot be measured readily, and histological sections cannot be relied upon to illustrate the thickness of the mucosa, as they intersect the folded surface at different angles in different places. Thus the advantages of early fixation may be more than offset by the impossibility of making good quantitative measurements. In this study severe degrees of postmortem change were avoided by using material from autopsies performed soon after death and by discarding those specimens which even then presented prominent digestive changes. Nothing has been noted to suggest that the measurements of mucosal area were affected in any way by the minor postmortem

12. Simpson, C. K.: Observations on Gastritis, Guy's Hosp. Rep. **84**:351, 1934. Aschner, P. W., and Grossman, S.: Gastritis and Duodenitis in Relation to the Ulcer Problem, Surg., Gynec. & Obst. **57**:334, 1933.

changes which had occurred. The observed mucosal thickness in histological sections and the estimations of the number of parietal cells, even though possibly influenced to a slight degree, are believed to be valid indices of the conditions which existed during life. There was no correlation between the estimated degree of postmortem change and the measured mucosal thickness or the number of parietal cells.

The thickness of the gastric mucosa cannot be measured accurately in gross specimens, even when postmortem digestion is not present, because the exact location of the base of the mucosa is not clearly visible in such material. Even the position of the tissue surface cannot always be sharply distinguished because of the frequent presence of tenacious mucus. It was concluded, therefore, that the most reliable measurement of mucosal thickness for the purposes of this study would be that made from histological sections. This thickness measurement was not equivalent to the thickness of the fresh mucosa, or even of the formalin-fixed tissue, as some shrinkage occurred during preparation of the sections, but it seems evident that measurements from histological sections can indicate true differences in different stomachs and in different parts of the same stomach.

Most anatomical studies have made a distinction between the two major zones of the gastric mucosa—the pyloric zone or antrum, and the fundic zone, or body—but it is still common practice to speak of the entire mucosa as a unit. The data presented in this paper indicate that not only do the two principal mucosal zones have a different appearance and function, but they also contribute to the total stomach size in different degrees in different persons. Observations in this laboratory and elsewhere have indicated differences in the occurrence of atrophic and inflammatory changes in the two zones,[13] thus providing further indication of their independent behavior. These differences should not be surprising, inasmuch as the difference in the structure and function of the two zones is as distinct as that of the large and small intestines. Disease of these two intestinal segments frequently follows quite different patterns.

The transition between the specific secretory zone and the non-acid-secreting portions of the stomach was abrupt in many cases, although in some there was an intermediate zone of varying width. This, however, was usually no more than a few millimeters broad, so it was ignored in the calculations based upon areas of the major mucosal zones.

The significance of the differences in stomach size illustrated in this paper has not been completely clarified. The fact that chronic gastritis of the fundic zone occurred in relatively more of the small stomachs may indicate a causal relationship, as the term "atrophic gastritis" implies, yet a good many small stomachs with relatively few parietal cells did not present much gastritis, and there were a number of large stomachs which presented similar gastritis.

The frequent association of duodenal ulcer and large stomachs suggests that the latter may be abnormal. These, however, are different only quantitatively from stomachs of average size. The degree of variance which was observed in adult human stomachs has not been encountered in numerous stomachs of rats, guinea pigs, and rhesus monkeys examined in this laboratory. That some of the size difference in

13. Faber, K.: Gastritis and Its Consequences, London, Oxford University Press, 1935. Cox, A. J.: Chronic Atrophic Gastritis: Identification of Different Varieties, J. Nat. Cancer Inst. **10**:523, 1949. Hebbel.[3]

stomachs of human adults represents acquired alterations is supported by the absence of such pronounced variations in infants' stomachs.[14] The relationship between stomach size and eating habits could not be studied in the material reported here because of the meager available information concerning these habits. No peculiarities of stomach size were observed in 30 obese persons.

The range of stomach size and number of cells in otherwise normal stomachs of different persons is comparable to that in other organs which have different functional states at different times. In muscles and endocrine glands, for example, there is a close relationship between size and functional activity. The possibility that some of the differences in gastric mucosal size may reflect functional differences is apparent. The predominant role of the acid-secreting zone of the mucosa in determining mucosal size gives support to this possibility, although the lack of sufficient antemortem studies of gastric secretion in the cases reported here prevented adequate comparisons of size and secretory activity of the same stomach. Tongen,[15] however, using rough measurements of the number of parietal cells, has reported a relationship between the number of cells and the secretory capacity of the stomach. This concept of the pathogenesis of gastric mucosal hyperplasia, which emphasizes the role of functional stimulation, does not require the participation of any such inflammatory reaction as is implied by the term chronic hypertrophic gastritis.

The high secretory activity of the stomach in patients with duodenal ulcer, recognized by most investigators, presumably reflects the presence of a large mucosa, and it follows that the large amount of secretory tissue in such stomachs may be a result of the disturbance responsible for the hypersecretion. Such a mechanism would be comparable to the prolonged stimulating effect of histamine, which in guinea pigs has resulted in an increase in the number of parietal cells.[7] If the active secretion of a large stomach is a factor in the development of chronic duodenal ulcer, it should be expected that persons subjected to this hazard for the longest time would show the greatest incidence of ulcer. That this actually occurs is suggested by the greater incidence of duodenal ulcers in old than in young persons whose stomachs contained many parietal cells. The sex difference in incidence of duodenal ulcer may be a reflection of the larger average amount of gastric mucosal tissue in males.

Shay [16] has expressed the belief that hypersecretion of the stomach may result from the presence of duodenal ulcer. This possibility cannot be refuted by the results of the studies reported here, although the absence of significant difference in stomach size in cases of healed duodenal ulcer contrasted with cases of active ulcer, together with the occurrence of some equally large stomachs in the absence of ulcer, fails to provide any support for a sequential role of the large stomach in cases of duodenal ulcer.

The possibility that obstruction near the site of a duodenal ulcer may cause stomach enlargement has been noted by Wangensteen and his associates [2] and by others. There is no reason to believe, however, that the bulky mucosa in the cases

14. Cox, A. J.: Unpublished data.

15. Tongen, L. A.: The Quantitative Relationship Between Parietal Cells and Gastric Acidity, Surgery **28**:1009, 1950.

16. Shay, H.: The Pathologic Physiology of Gastric and Duodenal Ulcer, Bull. New York Acad. Med. **20**:264, 1944.

of duodenal ulcer reported here was produced by obstruction. The lack of distinction between these and other large stomachs, the predominance of the fundic zone in the large stomachs, and the large number of parietal cells cannot be explained in this way.

The small average size of the gastric-ulcer-bearing stomachs was due in part to contraction of scars. Several stomachs, however, were small even when little scarring had occurred, and in none of the small stomachs was there a thick mucosa or pronounced surface nodularity to suggest that even prior to the scarring these stomachs had been comparable to the large stomachs in the duodenal ulcer cases. The difference in stomach size in gastric and in duodenal ulcer cases is consistent with the idea that there is a fundamental difference in pathogenesis of the two types of ulcer.

Many gastroscopists have described a verrucous, beaded, or cobblestone pattern of the mucosal surface as it is viewed during life. This is used by some as evidence of the presence of chronic hypertrophic gastritis; others distinguish between a so-called normal nodular pattern and one presumably due to gastritis. Among other reports, that of Jones [17] has illustrated a fairly uniform nodular mucosal pattern which was regarded as normal. It seems probable that some of the reported gastroscopically visible irregularities of the mucosal surface are identical with the above described type of nodular mucosal surface which is seen frequently post mortem in the fundic zone when the mucosa is thick, and it is possible that it may have been misinterpreted as evidence of inflammation. Schindler [18] has stated that chronic hypertrophic gastritis is much more frequent in the body than in the antrum of the stomach, and some of his illustrations present a mucosal pattern quite like that described in this report. The illustration of the histological appearance of "chronic glandular hypertrophic gastritis" by Schindler and Ortmayer [19] represents the feature under discussion. These authors have written: "One could think that this is a noninflammatory growth of the mucosa. . . ."

The reason for the growth of the mucosa in this nodular form is not known. Our observations do not confirm the thesis advanced by Hamperl [19] that the elevations are the result of contraction of smooth muscle exaggerated by fixation. The nodules were visible before fixation, no relationship to aberrant muscle cells was seen, and the mucosal area of nodular specimens, instead of being smaller, as should be expected in the presence of muscular contraction, was greatest in the stomachs with the most prominent mucosal nodules. It is possible that the irregularity is related to the mechanical characteristics of the mucosa which, if uniformly thick, would be less flexible than its thin counterpart. The numerous furrows which separate the surface nodules produce localized thin portions where folding may occur more readily than if the thickness were uniform.

The available evidence indicates that the variations in structure of the stomach described in this report represent changes of specific significance with respect to gastric physiology and pathology. It is probable, therefore, that as more is learned it will become desirable to make clinical measurements of stomach size. This might

17. Jones, F. A.: Discussion on Gastritis, Proc. Roy. Soc. Med. **38**:82, 1944.

18. Schindler, R.: Gastroscopy: The Endoscopic Study of Gastric Pathology, Chicago, University of Chicago Press, 1937.

19. Hamperl, H.: Die gröbere und feinere Gestaltung des Schleimhaut des Magendarmkanals in Abhängigkeit von seiner Muskulatur, Virchows Arch. path. Anat. **305**:432, 1939.

be achieved through direct measurement of capacity or the study of x-ray silhouettes under controlled conditions. The degree of nodular irregularity of the mucosal surface in the fundic zone as seen at gastroscopy should provide another index of stomach size. Observing the size and number of mucosal folds is of potential clinical usefulness in this connection, since the prominence of these folds in the contracted stomach is related to the mucosal area.

## SUMMARY AND CONCLUSIONS

The area of the gastric mucosa is a satisfactory index of stomach size since it remains nearly constant as the capacity of the organ changes over a wide range.

Stomach size varies more than threefold in different persons and is not closely related to the size of other organs. Most of the difference in mucosal size is due to differences in quantity of the specific secretory portion of the gastric mucosa, which in large stomachs usually contains large numbers of parietal cells. A bulky mucosa is presumably a manifestation of hyperplasia, probably related to hypersecretion.

Chronic duodenal ulcer or scar is usually associated with a large stomach which has a large amount of secretory mucosal tissue. The possibility that this may be of etiological significance is discussed.

Stomachs bearing gastric ulcers or scars, on the other hand, have a smaller average size than non-ulcer-bearing stomachs.

The size of the mucosal folds and the degree of surface nodular irregularity of the gastric mucosa are suggested as indices which might be used to estimate stomach size during life.

Miss Virginia Barnes, Miss Murrell Green, and Mrs. Helen Treinen gave helpful assistance.

# OSTEOARTHROPATHY IN DOGS

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BOSTON

THE FIRST description of pulmonary osteoarthropathy in man [1] has been followed by reports of similar lesions in animals (horse,[2] cow,[3] sheep,[4] dog,[5] cat,[6] fowl,[7] lion,[8] and deer [9]). Since so few pathological studies of the lesions in dogs have been reported, this study of four cases is presented.

From the Department of Pathology, Angell Memorial Animal Hospital.

This study was aided by a grant from the Swiss Academy of Medical Sciences.

Present address of author: Veterinary Pathological Institute, University of Zurich, Zurich, Switzerland.

1. Bamberger, E.: Über Knochenveränderungen bei chronischen Lungen- und Herzkrankheiten, Ztschr. klin. Med. **18**:193, 1890-1891. Marie, P.: De l'ostéo-arthropathie hypertrophiante pneumique, Rev. méd., Paris **10**:1, 1890.

2. (*a*) Wirth, D., and Pommer, A.: Akropachie beim Pferde, Arch. wissensch. u. prakt. Thierh. **63**:230, 1931. (*b*) Liénaux, M.: Remarque au sujet de la nature de l'ostéo-arthropathie hypertrophiante: Un cas de co-existance de l'ostéopériostite diffuse et la tuberculose chez le cheval, Ann. méd. vét. **58**:313, 1909. (*c*) Cotchin, E.: Marie's Disease Associated with Tuberculosis in a Horse, Brit. Vet. J. **100**:261, 1944; Marie's Disease (Hypertrophic Pulmonary Osteo-arthropathie): Two Further Cases in the Dog, ibid. **100**:45, 1944. (d) von Guoth, G. A.: Über Akropachie beim Pferde, Deutsche tierärztl. Wchnschr. **48**:354, 1940.

3. Laszlo, F.: Akropachie beim Rind, Deutsche tierärztl, Wchnschr. **37**:776, 1929.

4. Nieberle, K., and Cohrs, P.: Lehrbuch der speziellen pathologischen Anatomie der Haustiere, Ed. 2, Jena, 1949.

5. (*a*) Wissing, E. G., and Weisz, L.: An Unusual Case of Pulmonary Osteoarthropathy, Am. J. Roentgenol. **50**:527, 1943. (*b*) Wirth, D.: Periostische Veränderungen am Skelett bei Tuberkulose des Hundes, Ztschr. Tuberk. **24**:393, 1921. (*c*) Stang and Wirth: Tierheilkunde und Tierzucht, Berlin, Urban & Schwarzenberg, 1926, Vol. 1. (*d*) Cordy, D. R., and Dinsmore, J. R.: Pulmonary Hypertrophic Osteoarthropathy (Marie's Disease), North Am. Vet. **31**:30, 1950. (*e*) Poley, P. P., and Taylor, J. S.: Hypertrophic Pulmonary Osteoarthropathy Associated with a Bronchiogenic Giant Cell Tumor in the Left Lung of a Dog, J. Am. Vet. M. A. **100**:346, 1942. (*f*) Rumney, W. J., and Schofield, F. W.: Pulmonary Hypertrophic Osteoarthropathy (Marie's Disease), Canad. J. Comp. Med. **14**:385, 1950. (*g*) Schlotthauer, C. F., and Millar, J. A. S.: Hypertrophic Pulmonary Osteoarthropathy in Association with Pulmonary Neoplasms in Dogs, J. Am. Vet. M. A. **119**:442, 1951.

6. Houdemer, Genevray, Dodero, and Farinaud: Contribution à l'étude des ostéopathies hypertrophiantes des carnivores domestiques, Bull. Acad. vét. France **6**:439, 1933.

7. Kitt, T.: Die Akropachia ossea der Hunde und Hühner, München, tierärztl. Wchnschr. **77**:554, 1926.

8. Ball, V., and Lombard, C.: Osteoarthropathie hypertrophiante pneumique chez les fauves en captivité: Premier cas de syndrome de Pierre Marie chez une lionne, Bull. Acad. méd., Paris, **95**:16, 1926.

9. Ball, N.: Ein Fall von multipler Exostosenbildung bei einem Hirsche (Zur Frage über die Osteoarthropathie hypertrophiante Marie), Arch. wissensch u. prakt. Thierh. **60**:235, 1929.

In man the clubbing of fingers and toes usually represents the initial stages of pulmonary osteoarthropathy and is of great diagnostic significance. In animals, on the other hand, alterations of the phalanges seem to be a very inconstant sign. In animals, as in man, the onset of the disease is painless, and the early stages usually are not noticed. Veterinarians, therefore, see for the most part the more advanced stages, in which symmetrical thickening of the extremities is typical.

## PRESENT INVESTIGATION

*Material.*—This study is based on material obtained from four dogs on which autopsies were done in the Department of Pathology at the Angell Memorial Animal Hospital in Boston. The histories and clinical, roentgenographic, and autopsy findings are summarized in the accompanying table.

*Observations.*—In these cases, the bones affected, in order of decreasing frequency, were the radius, ulna, tibia, fibula, metacarpals and metatarsals, humerus, femur, and the first and second phalanges. In no case was a terminal phalanx altered. The scapulae were slightly affected in Case 3. When metacarpal or meta-

*Summary of Case Histories, and Clinical, Roentgenographic, and Autopsy Findings in Dogs*

| Case | Anamnesis | Primary Lesions | Roentgenographic Involvement | Clinical Signs |
|---|---|---|---|---|
| 1. | Gordon setter, M., 4 yr.; respiratory disturbances | Bronchogenic carcinoma | All long bones with exception of terminal phalanges; acute stage | Clubbing |
| 2. | Cocker spaniel, F., 11 yr.; thickening of limbs over 1 month; respiratory disturbances, anorexia, loss of weight | Fibrosarcoma of pleura, metastasizing to lung | All long bones with exception of terminal phalanges and femora; acute stage; prominent proliferations | None noted |
| 3. | Cocker spaniel, F., 10 yr.; thickening of limbs over 6 to 8 months; dyspnea | Bronchogenic carcinoma | All long bones with exception of terminal phalanges; carpal and tarsal bones not altered; chronic stage | Enlargement of nails |
| 4. | German shepherd, F., 10 yr.; loss of weight over 5 months; dyspnea | Adenocarcinoma of perianal gland with multiple metastases to lungs | Slight, localized | None noted |

tarsal bones were involved, the medial aspect of the second and the lateral surface of the fifth metatarsal showed the greatest reaction. No lesions of the axial skeleton were found.

Study of cross sections of tubular bones, in different sectors of the same circumference revealed variations in the extent and rate of progress of the bony alterations. In the same animal, lesions were usually in approximately the same stage of development; yet there were occasionally striking differences in related bones, such as the tibia and fibula. According to the rate of development of proliferations, acute and chronic forms were distinguishable, but in either form the intensity of the process varied from sector to sector (Fig. 3).

(*a*) Acute Form: Deposition of large thicknesses of subperiosteal bone characterized this type of lesion. The new bone was covered with a thickened vascular periosteum. There were occasional small islands of round cells between the two periosteal layers, but active inflammatory changes were minimal or absent. The original external layer of the cortex was overlaid by periosteal bone consisting of numerous trabeculae generally situated perpendicularly to the cortical bone. In some areas concomitant rapid bone deposition was accompanied by bony resorption, as was evidenced by large numbers of osteoclasts. In these spaces vascular con-

Fig. 1 (Case 3).—Roentgenogram of the left foreleg, showing extensive alterations in the distal part of the radius, ulna, and metacarpal bones, with marked thickening of the extensor and flexor surfaces. The humerus and the first and second phalangeal bones show slight alterations.

Fig. 2 (Case 2).—Roentgenogram of the forelegs, showing moderate clubbing of the toes and symmetrical periosteal proliferation. The process is marked in the radius, ulna, first, second and fifth metacarpal bones and in the first and second phalanges.

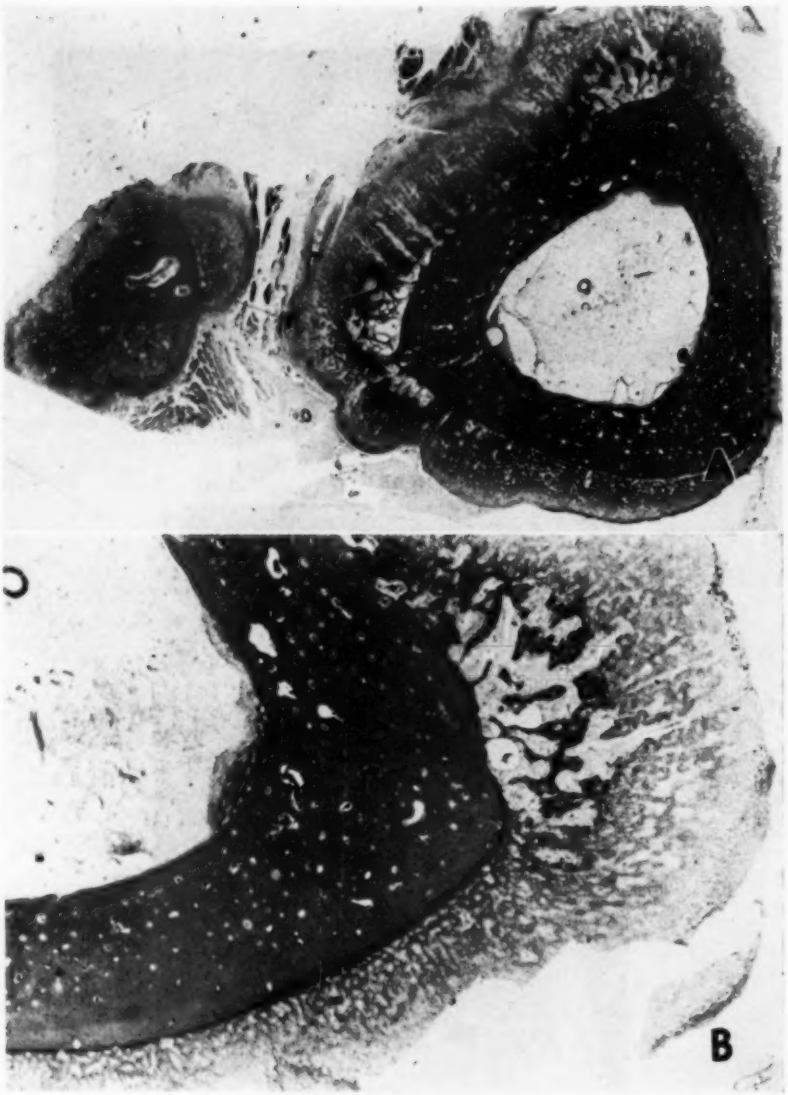


Fig. 3 (Case 2).—*A*, cross section of tibia and fibula. Note the production of an irregular periosteal bone girdle, consisting of perpendicular trabeculae. In the basal layers of the two sections there are osteoclasis and transformation of the primary periosteal to a lamellar bone. In the tibia, there is formation of an endosteal protuberance, built up by tangential trabeculae. Ossification in the deeper layer of the periosteum is more advanced than in the periphery. *B*, cross section of tibia, showing osteoclasis with enlarging of the intertrabecular spaces. In the same sector localized endosteal proliferation is present.

gestion was marked, and often transformation of the primitive connective tissue into active, hematopoietic marrow had begun.

Simultaneously the primary fibrillar bone underwent resorption and replacement by lamellar bone. The new subperiosteal bone sheath and the original compacta were thus connected by a few slender trabeculae. The original cortex showed only small areas of bone resorption at its surface and at the periphery of the Haversian canals. Endosteal bone production was not very extensive but was present and active in several of the bones examined. It was prominent in those sectors in which periosteal reaction was most severe. In most other sectors, the endosteum was normal with no signs of hyperactivity.

The primary marrow cavity contained either fatty or active hematopoietic marrow.

(*b*) Chronic Form: This form was characterized by progressive maturation of the subperiosteal bone. The trabecular framework of the subperiosteal bone was replaced by layers of lamellar bone which overlaid the original cortex. The periosteum appeared as a rather thin membrane, in which the cambium was thin or even absent. The loss of its bistratal structure characterized the inactive stage of the disease. In these cases the endosteum was unaltered.

In several bones two to seven laminae constituted evidence of a cyclical deposition of periosteal bone. These laminae were usually narrow. In the same cross section there were some sectors with seven laminae and some sectors with only two. The sectors of the compact bone which were not distinctly thickened often contained small Haversian spaces filled with fatty marrow, while thickened sectors seemed to have larger Haversian spaces.

## COMMENT

The development of skeletal changes secondary to lesions of the lungs gave rise to the term "pulmonary osteoarthropathy." However, some cases of idiopathic or primary osteoarthropathy have occurred in men and animals.[5a] The term "secondary osteoarthropathy" would seem, therefore, to be more appropriate. Other terms have been suggested, in view of the subordinate character of the joint changes, e. g., diffuse osteoperiostitis, osteopathia, acropachia, and pachymelia. These have not been generally accepted.

The chain of events which follows the development of a primary lesion of the lung and leads to secondary osteoarthropathy is unknown. The material here presented brings out the following points.

The variations in extent of the proliferations in the different bones of an animal and even in the same cross section could scarcely be accounted for on the basis of a generalized metabolic disorder. The deposition of subperiosteal bone altered the mechanical function of the cortex. In turn, local mechanical factors, such as muscle attachments and pressures, influenced the extent of subperiosteal bone formation. The newly formed subperiosteal fibrillar bone gradually assumed the functions of the original cortex [9] and was transformed into lamellar bone.

In one of our cases (Case 3) a basophilic adenoma of the pituitary gland was found. This neoplasm is very rare in animals.[10] No coincidence of pituitary

10. Brandt, A. J.: Über Hypophysenadenome bei Hund und Pferd, Scandinav. vet. tidskr. **30**:875, 1940.

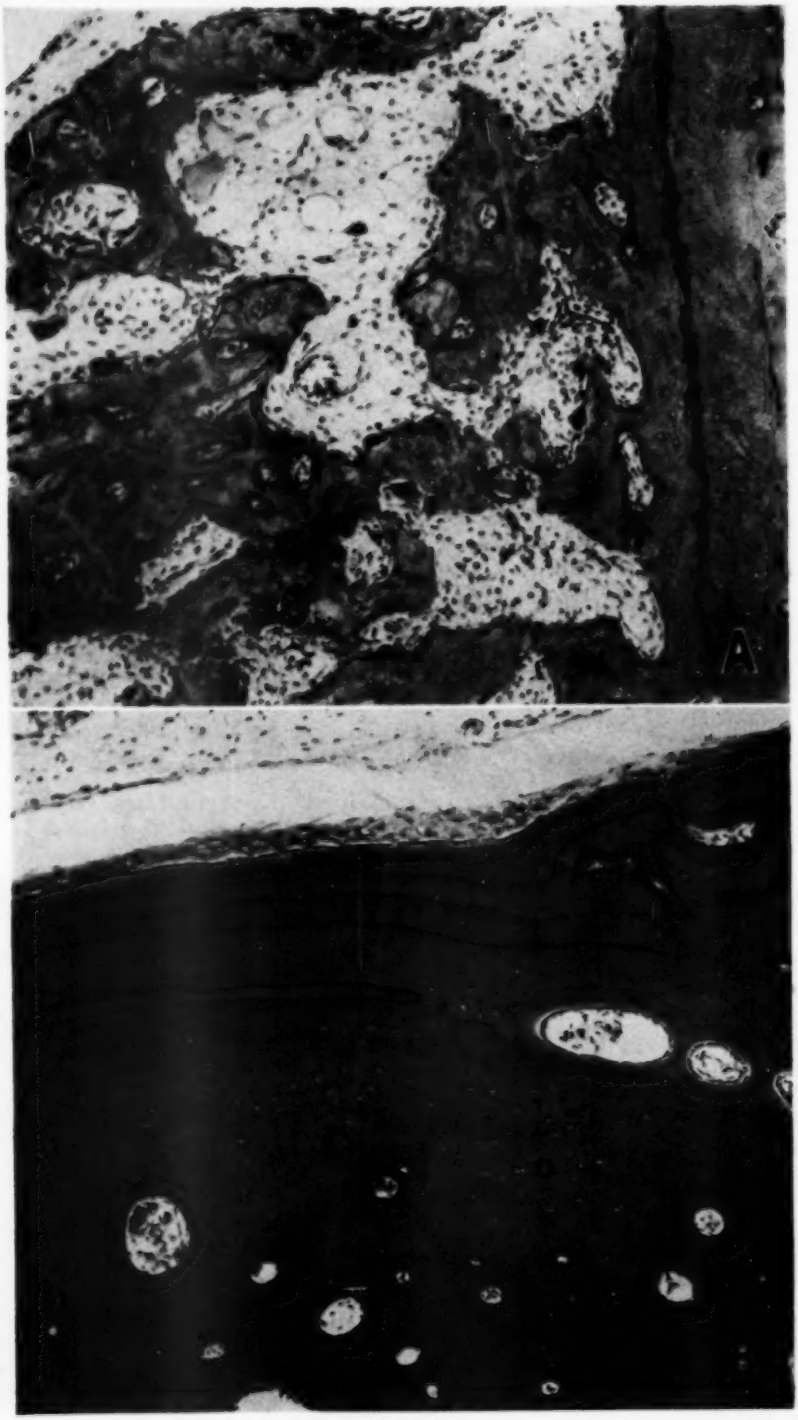


Figure 4

*(See legend on opposite page)*

adenoma and secondary osteoarthropathy has been reported, and the few reports of such neoplasms do not mention alterations in the tubular bones. Therefore we are inclined to regard the basophilic adenoma in our case as an incidental finding. The available material did not permit any statistical conclusions as to the sites most frequently affected. However, our experience confirmed the observations of Gall and his associates [11] that the extensor surfaces of the long bones and the membranous and tendinous attachments were the sites of greatest involvement.

In the German and French literature, secondary osteoarthropathy in animals is reported mostly as a sequela of tuberculosis.[12]

Although a few cases have been associated with other infections,[13] modern veterinary literature accents the important role of primary or secondary tumors of the lung.[14]

The primary etiological factors most frequently reported are tuberculosis and neoplasm of the lower respiratory tract.

## SUMMARY

Four cases of secondary osteoarthropathy in dogs, ranging in age from 4 to 11 years, are described. The osteoarthropathy was associated with lung tumors, of which two were bronchogenic carcinoma, one a fibrosarcoma of the pleura with metastases to the lungs, and one an adenocarcinoma of the perianal gland with multiple metastases to the lungs. The histological changes were classified as slow or rapid in development. In the same animal the bones varied considerably in the degree of involvement, and in the same bone frequently in one portion of the cortex there were minimal changes while in another portion considerable subperiosteal bone was deposited.

The acute form was characterized by active subperiosteal and endosteal bone deposition and by a gradual transformation of the primary fibrillary bone to lamellar structures, combined with a transformation of the intertrabecular tissue to an active bone marrow. The chronic form showed minimal alterations of the periosteum with amalgamation of the subperiosteal bone formation with the cortex. The cyclical new deposition of bone produced cement lines, varying in number from sector to sector.

11. Gall, E. A.; Bennett, G. A., and Bauer, W.: Generalized Hypertrophic Osteoarthropathy: Pathologic Study of 7 Cases, Am. J. Path. **27**:349, 1951.

12. Ball and Lombard.[6] Liénaux.[2b] Houdemer and others.[6] Wirth.[5b] Stang and Wirth.[5c] Cotchin.[2c] Gall and others.[11]

13. Ball.[9] Cordy and Dinsmore.[5d]

14. Wissing and Weisz.[5a] Laszlo.[3] Poley and Taylor.[5e] Rumney and Schofield.[5f] Schlotthauer and Millar.[5g]

### EXPLANATION OF FIGURE 4

Fig. 4.—*A* (Case 2), osteoclasis in the basal layer with lacunar resorption of the peripheral cortex. The intertrabecular connective tissue is in an initial stage of transformation into cell-rich marrow. *B* (Case 3), cross section of phalangeal bone with varying number of broken, densely stained cement lines, showing the cyclical deposition of bone substance.

# PULMONARY REACTION TO BARIUM SULFATE IN RATS

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D. P. WALLACH
AND
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DURING a recent examination of the pharynx and esophagus of a patient by means of a barium swallow, it was noted at fluoroscopy and on subsequent roentgenography that small amounts of barium sulfate suspension had entered the lungs (Fig. 1). Since aspiration of this nature has previously been observed here upon several occasions, it is probable that other radiologists have made similar observations. While radiologists in this hospital (personal communication) considered that such barium had no ill effect on the lung, it seemed worth while to conduct an experimental study in order to determine the detailed histological reaction of rat lung following the introduction of barium sulfate suspensions.

## LITERATURE

Fishel (1923) [1] reported a case of accidental introduction of a watery solution of barium sulfate into the lungs of a patient suffering from a tracheoesophageal fistula. Most of the barium remained present after four months but apparently had no ill effect. Ross Golden (1929) [2] has also made reference to the finding of barium sulfate in the lungs of patients. There has, however, been little done on the experimental side of this problem. Arrigoni (1933) [3] made a clinical and roentgenological study on a type of pneumoconiosis occurring in barium miners. This he followed up with an experimental study in rats and rabbits (1933).[4] The animals were given endotracheal injections of a suspension of three different grades of material obtained from barium mines. All these samples contained between 85 and 90% of barium, though one contained in addition 2.5% of silica. In addition, other animals were injected with a suspension of pure barium sulfate. In each case Arrigoni obtained similar histological appearances in the lungs. Many of the changes he described were similar to those that we observed, though he does not mention the marked

From the Departments of Pathology and Radiology, University of Wisconsin Medical School.

* This work was performed under the auspices of a Dorothy Temple Cross Research Traveling Fellowship.

1. Fishel, C. R.: J. A. M. A. **80**:102, 1923.

2. Diagnostic Roentgenology, edited by R. Golden, Baltimore, Williams & Wilkins Company, 1934 (4th printing), Vol. 1, pp. 154-155.

3. Arrigoni, A.: Clin. med. ital. **64**:299, 1933.

4. Arrigoni, A.: Med. d. lavoro **24**:461, 1933.

polymorph reactions seen in the early stages in our animals. We, however, did not observe the fibrosis and tubercle formation that he describes, and the reactions that we observed seemed so mild and insidious that it would be unlikely for much fibrosis to develop even if there were considerably longer intervals in which to do so.

## METHODS AND MATERIALS

The material was instilled directly by tracheal intubation, in order to avoid any surgical procedure that might complicate the ultimate findings.

The animals used in this study were male rats of the Sprague-Dawley strain, weighing between 250 and 300 gm. They were first lightly anesthetized with an intraperitoneal injection of 1 cc. of thiopental (pentothal®) sodium solution containing 6 mg. per cubic centimeter. The

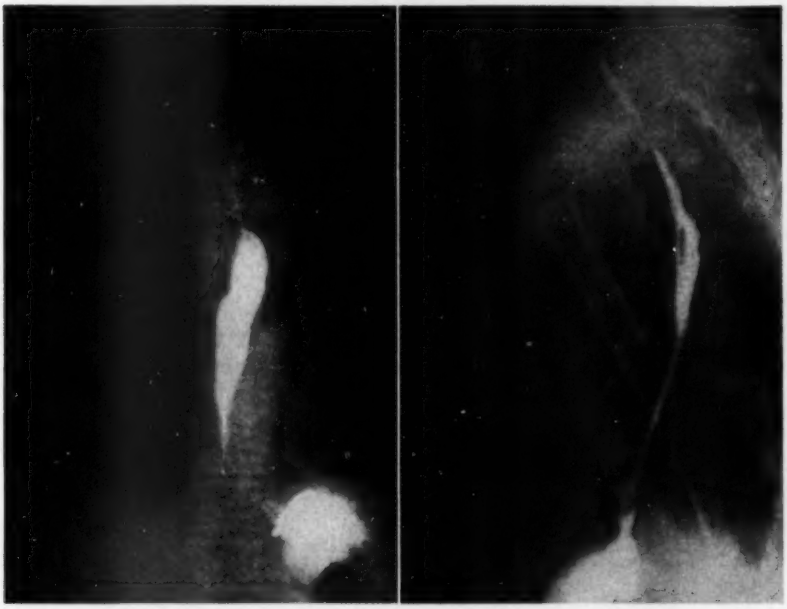

Fig. 1.—*A*, posteroanterior chest roentgenogram of patient with carcinoma of the breast with pulmonary, pleural, and mediastinal metastases; there is a small quantity of aspirated barium suspension outlining the walls of the trachea and right main bronchus. *B*, lateral chest roentgenogram from the same patient, showing radiopaque material in the right main bronchus and branches.

animal was tied in the supine position to a rat board by means of rubber bands on all four extremities. A fifth band, fitted over the front teeth, immobilized the head. With the board placed upright on a table and supported against the chest of the operator, the rat was thus held in a vertical position facing away from the operator. An assistant with a pair of forceps pulled the tongue of the animal as far out and to one side as possible, while the operator placed the tip of the tracheal catheter just at the base of the tongue. By pressing the needle ventrally it was possible to approximate its tip to the laryngeal opening, thus sealing off the airway. At this point the animal always showed signs of asphyxia, whereupon the injection was made. The needle was immediately removed, but the tongue was not released until the animal had aspirated most of the material. The rat was then removed from the board and usually had recovered consciousness within an hour. For injection, a 1 cc. tuberculin syringe was attached to a 15 gauge 2 in.

(5 cm.) needle, the point of which had been replaced by a blunt lead bulb. The preparation of barium sulfate used was a commercial one named "veriopaque," distributed by the General Electric X-ray Corporation, Milwaukee. While in general the animals survived this procedure well, a small number died almost immediately from tracheal or bronchial obstruction. Occasionally a small quantity of the barium suspension overflowed into the esophagus and thence to the stomach, but it gave rise to no apparent ill effect and was detected on roentgenographic examination.

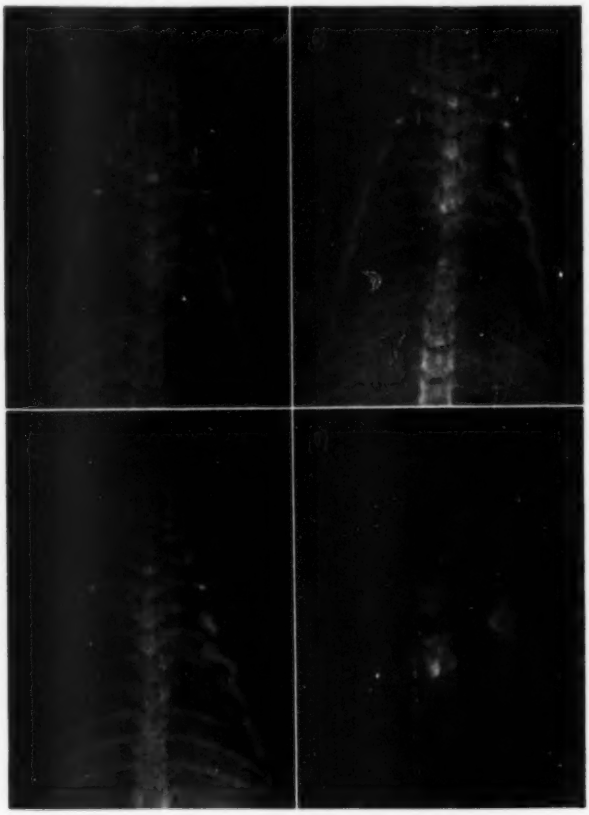

Fig. 2.—*A*, anteroposterior chest roentgenogram of a rat taken immediately before injection of barium sulfate suspension; *B*, anteroposterior chest roentgenogram of the same animal taken immediately after the barium injection; *C*, anteroposterior chest roentgenogram taken 15 days after the injection; *D*, roentgenogram of the lungs of the same animal after their removal from the body.

Roentgenograms were taken immediately prior to and after injection. This was accomplished with the animal still in position by removing a cut-out portion of the rat board and placing the apparatus on an x-ray casette. Further roentgenograms were taken of the animals before killing and of the lungs after removal from the body (Fig. 2). These were of value in detecting the lobes which contained barium suspension.

After some initial trials, 18 animals were used in this experiment. Of these, two died within two hours of the injection. The remainder were killed at varying intervals, of 30 minutes; 2, 12, 24, 48, and 72 hours, and 7, 15, 30, 94 and 126 days by a lethal intraperitoneal injection of thiopental sodium. Autopsies were performed immediately, and the lungs removed *in toto* with the minimum of handling. The lungs, together with the spleen, and portions of liver and kidney were fixed in 10% formalin. When fixation had taken place, sections were made from barium containing whole lobes, and by cutting through the hilus in most cases one or more lymph nodes were included in the sections.

### HISTOLOGICAL EXAMINATION

In the first instance, we employed a number of staining procedures in addition to the routine hematoxylin and eosin stain. These procedures included Weigert's elastin with Van Gieson stain, and the periodic Schiff technique. We discovered that barium suspension could be very easily identified histologically in hematoxylin-and-eosin-stained sections by using a dark-field microscope. This method proved to be ideal, though it was subsequently found that a similar and adequate identification could be made by tilting the mirror of an ordinary light microscope until the light was almost cut out. This enabled us to identify the barium suspension with great rapidity under the low power of the microscope and thus to obtain an accurate idea of its disposition.

In attempting to classify the histological appearances, we observed whether free particles were present and how they were disposed in the lung structure; whether the particles had been ingested and whether by polymorphonuclear leucocytes or by mononuclear cells; whether there was any proliferative action on the part of the lung tissue.

In the two animals which died within two hours of the injection, all the particles were lying free and were seen in large bronchi and bronchioles. In our examination of the remaining animals we were able to identify four more or less clearly defined histological patterns.

*Stage of Initial Inflammatory Reaction* (Fig. 3).—This bore a resemblance to an acute inflammation, in that large numbers of polymorphonuclear leucocytes could be seen in the areas of lung containing barium suspension. These cells filled many of the alveolar spaces and smaller bronchioles and contained considerable numbers of foreign particles. Free particles of suspension were occasionally seen. Mononuclear cells were few, and there was little evidence of the fluid exudative changes so characteristic of acute inflammation. These appearances were well developed at 12 hours, but by 24 hours the polymorphonuclear cells were showing degenerative changes, such as loss of cell outline, and were becoming considerably reduced in numbers.

*Stage of Mononuclear Infiltration* (Fig. 4).—This stage was seen in all animals killed between 48 hours and 15 days. Under the low power, consolidated areas could be seen which contained barium suspension. These were composed of large numbers of mononuclear cells packed with particles of suspension. These cells appeared quite healthy, as the cell outline was well defined and the nucleus showed no abnormality. The epithelium lining the smaller bronchioles had disappeared and was replaced by phagocytic mononuclear cells which appeared to be lining the lumen (Fig. 5). There was a surprising lack of reaction on the part of other cells, such as lymphocytes and plasma cells.

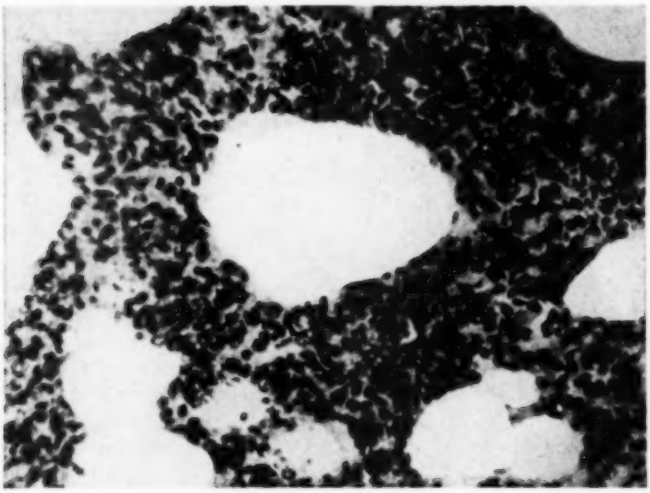

Fig. 3.—Section of rat lung 12 hours after injection, showing extensive polymorphonuclear infiltration of the area around an alveolar duct. Hematoxylin and eosin; × 175.

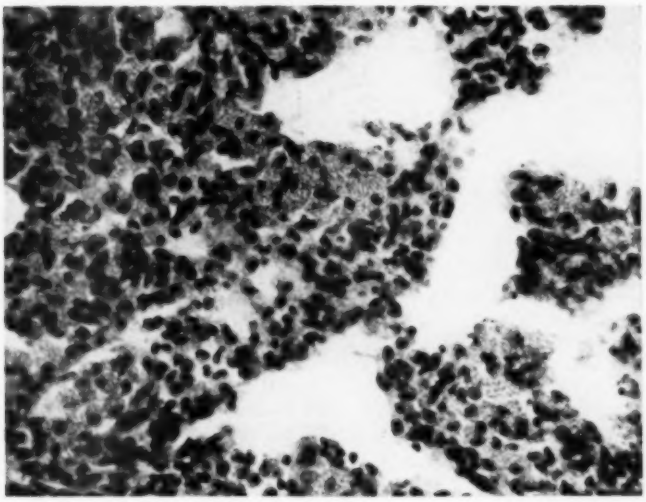

Fig. 4.—Section of rat lung three days after barium injection, showing granular refractile material within mononuclear cells. Only a few polymorphonuclear cells remain. Hematoxylin and eosin; × 175.

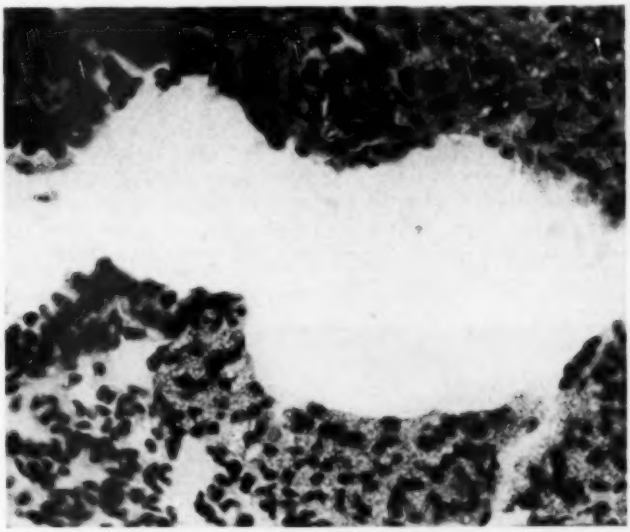

Fig. 5.—Section through respiratory bronchiole showing large numbers of mononuclears containing barium suspension. Note the replacement of the lining epithelium by these cells. Fifteen days after injection. Hematoxylin and eosin; × 300.

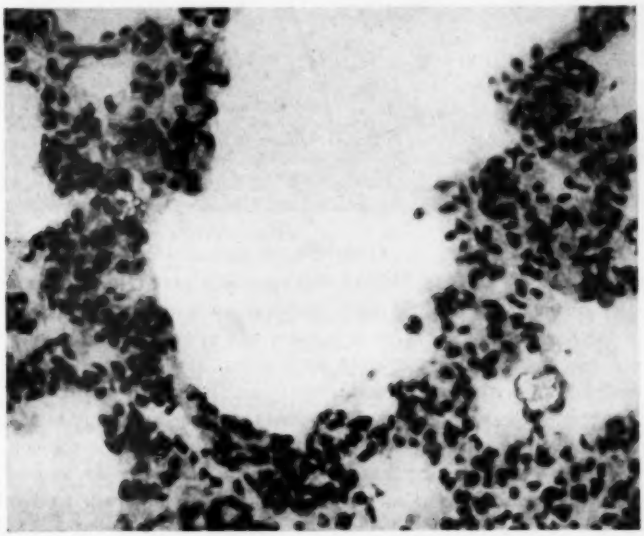

Fig. 6.—Section of lung 30 days after injection, showing refractile bodies present both in the alveolar lumen and in the interstitial tissue. Hematoxylin and eosin; × 175.

*Stage of Mononuclear Disintegration* (Fig. 6).—By the 30th day the mononuclear cells were still present in large numbers in the interstitial tissue but had undergone marked change of appearance. Many had lost their cell outline and were fused together. Others had ruptured and liberated the barium salt, which could be seen aggregated into refractile masses.

*Stage of Tissue Reaction* (Fig. 7).—In sections taken at 94 and 120 days, a mild tissue reaction was seen. The lung tissue looked for the most part normal, except where some small solid areas were seen in relation to the refractile masses referred to in the last stage. Here a few lymphocytes and mononuclear cells could be seen, and there was evidence of early giant cell formation. There was no suggestion of fibrosis as yet in these areas.

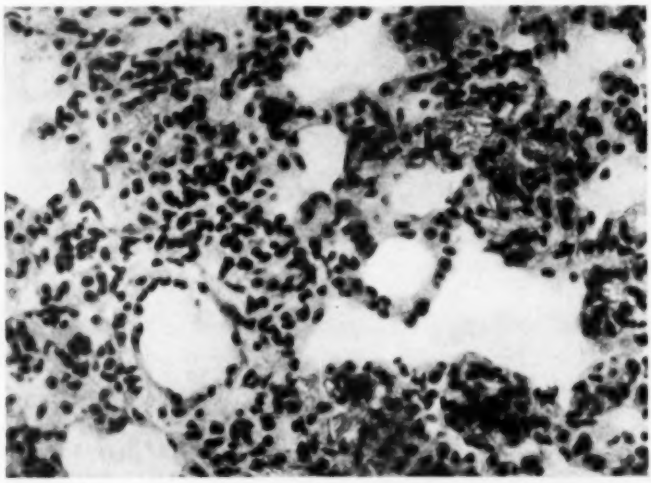

Fig. 7.—Section of lung 16 weeks after injection, showing refractile bodies with mild surrounding tissue reaction and early giant cell formation. Hematoxylin and eosin; × 175.

## COMMENT

It has been stated (Sullivan, 1934) [5] that barium is removed from the lungs by coughing, by ciliary action, and by macrophages carrying the material to the lymph nodes. In the present study, our main interest was in the last means of disposal. We were consequently rather surprised to find that the vast majority of lymph nodes examined did not contain barium suspension. In one rat killed at 126 days, some mononuclear cells containing barium were seen, but these were relatively few (Fig. 8). Examination of the spleen, liver, and kidneys showed no relevant abnormality. It would therefore appear that once barium sulfate enters the lung substance its removal by mononuclear transportation is in some way inhibited. Certainly once these cells had ingested barium they appeared rather inert, and it is likely that barium does exert some toxic effect, in view of the degenerative changes observed

5. Sullivan, S. J.: J. A. M. A. **103**:337, 1934.

both in the polymorphonuclears and the mononuclears which contained the salt and also in the respiratory epithelium lying adjacent. We attempted to investigate this "paralysis" of mononuclear cells by injecting other salts. For this purpose we injected four series of 10 rats with 7.5% suspensions of salts of varying molecular weights, and employed ferric oxide, magnesium carbonate, and barium carbonate. These suspensions were made in 1% tragacanth in order to make them comparable with the suspension used in the first experiment. At the same time, we confirmed our previous findings by doing a fresh series of ten rats with a similar suspension of barium sulfate. The injections of ferric oxide, barium carbonate, and magnesium carbonate caused intense acute inflammatory reactions, and consequently the lesions were not comparable with those produced by the comparatively inert barium sulfate.

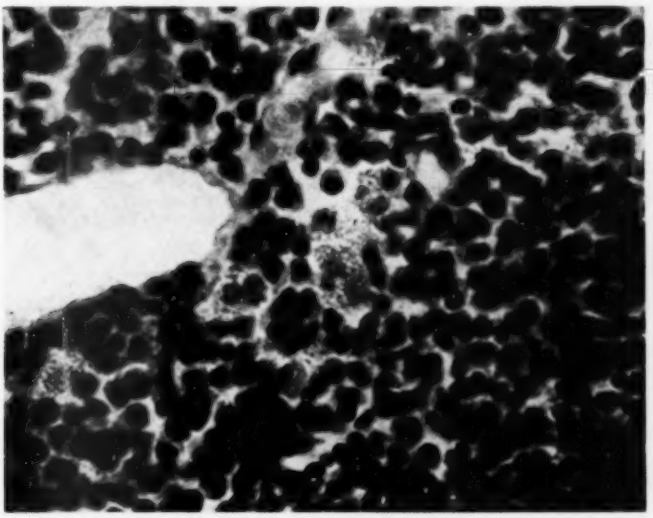

Fig. 8.—Section of lymph node showing a few mononuclear cells containing particles of barium suspension. From an animal killed 126 days after injection. Hematoxylin and eosin; × 700.

The vehicle in which the barium sulfate was suspended gave a positive reaction with the periodic Schiff technique. We therefore concluded that we should inject five rats with a similar aqueous suspension of barium sulfate to make certain that the changes we had observed were really due to the barium salt itself. In this series, the changes were in every way similar to those in the previous one, except that the refractile masses of barium sulfate appeared somewhat sooner. This may well have been accounted for by the fact that the particles in this suspension were not so fine as in the commercial preparation, a fact which was immediately apparent to us as we examined these suspensions with the microscope. We had also noted in our studies, as did Arrigoni (1933),[4] that small particles of suspension were seen intracellularly while larger ones were seen free in the tissue.

## CONCLUSIONS

On the basis of these observations it is concluded that barium sulfate is a relatively inert substance in the tissues and gives rise to only a very mild reaction. At first the suspension is taken up by polymorphonuclear leucocytes, which subsequently degenerate and liberate the suspension, which is then taken up by mononuclear cells. It is likely that toxic action of the salt accounts for the degeneration of the polymorphonuclears as well as for the similar change which overtakes the mononuclears at a later date. When the barium is liberated from these cells, it becomes inspissated into refractile masses, though we have no explanation as to why this occurs. The toxic effects of the barium salt may also be seen in the respiratory epithelium, which probably undergoes degeneration and subsequently regenerates. The amount of tissue reaction is slight, and giant cell reaction of the foreign body type commences after about 90 days. Finally, although barium sulfate if present in quantity cannot be completely removed, it is likely to give rise to little fibrosis ultimately.

## SUMMARY

An experimental study of the reaction of rat lung to the endotracheal injection of barium sulfate suspensions is described.

Stages of this reaction which are moderately clear-cut have been found, although the changes are mild and insidious.

An attempt to elucidate the reason for the persistence of barium sulfate in the lungs by comparison with the behavior of other salts failed, owing to an intense inflammatory reaction produced by these substances.

Dr. D. M. Angevine and Dr. L. W. Paul provided histological and roentgenological facilities and gave helpful advice in this study; Miss R. Campbell and Mrs. C. Richter provided much valuable technical assistance in histological methods, and Mr. Homer Montague took the photographs and photomicrographs.

# OBSERVATIONS ON SPECIFICITY OF SCHULTZ HISTOCHEMICAL TEST FOR DEMONSTRATING CHOLESTEROL IN TISSUE

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BIRMINGHAM, ALA.

SCHULTZ,[1] in 1925, described a modification of the Liebermann-Burchardt test that could be used to demonstrate cholesterol in tissue section. Since that time this method has been widely used.

A number of other methods for demonstrating cholesterol in tissues are found in the literature.[2] Several chemical reactions of cholesterol that produce color and might be adapted to tissue work have been described also.[3] On trying these different methods and comparing them with the Schultz test, the latter was found to be more satisfactory.

There is considerable doubt, however, regarding the specificity of the Schultz test. It has been shown that compounds other than cholesterol and cholesterol esters will produce color in the Liebermann-Burchardt reaction.[4] The color produced by some compounds is indistinguishable from that produced by cholesterol. It is

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important, therefore, to establish whether or not the Schultz test will produce a color reaction with other compounds in tissue sections which might be confused with that produced by cholesterol. The following experiments were done in an attempt to clarify this question.

## METHODS AND MATERIALS

Twenty-four chemical compounds were tested. Some were structurally related to cholesterol. Others had been found by previous investigation to produce color reactions with the

*Color Reactions of Twenty-Four Chemical Compounds with the Liebermann-Burchardt Test and with the Schultz Test*

| Test Reactions | Liebermann-Burchardt Reaction in Test Tube | Liebermann-Burchardt Reaction on Tissue | Schultz Test |
|---|---|---|---|
| **Control Group** | | | |
| Aorta (acetone-extracted) | ..... | Faint purple | Faint purple |
| Aorta (atherosclerotic) | ..... | Blue-green 4+ | Blue-green 4+ |
| Adrenal cortex | ..... | Blue-green 4+ | Blue-green 4+ |
| Cholesterol (Merck) | Blue-green 4+ | Blue-green 3+ | Blue-green 3+ |
| Cholesterol palmitate | Blue-green 4+ | Blue-green 3+ | Blue-green 3+ |
| Cholesterol oleate | Blue-green 4+ | Blue-green 3+ | Blue-green 3+ |
| **Group Structurally Related to Cholesterol** | | | |
| Testosterone | Eosin red | Eosin red | Yellow-red |
| Progesterone U. S. P. | Light yellow | Light yellow | Light brown |
| Estradiol U. S. P. | Light yellow | Light yellow | Light yellow |
| Estrone U. S. P. | Eosin red | Eosin red | Yellow-brown |
| Dehydroisoandrosterone acetate | Deep red | Moderate red | Deep red |
| Desoxycholic acid | Orange | Light yellow | Light purple |
| Cholic acid | Yellow-red | Light yellow-red | Yellow-brown |
| **Liebermann-Burchardt-Positive Group** | | | |
| Ergosterol | Blue-green 4+ | Blue-green 3+ | 0 |
| Carotene (90% beta and 10% alpha) | Blue-green 4+ | Blue-green 4+ | Blue-green 4+ |
| Vitamin A (alcohol) | Blue-purple | Blue-purple | Blue-purple |
| Linoleic acid | Blue-green 4+ | Blue-green 3+ | 0 |
| **Liebermann-Burchardt-Negative Group** | | | |
| Eugenol | Dark red-brown | Red-brown | Light brown |
| Terpineol | Violet | Light violet | Light purple |
| Anethole | Deep red | Light blue | Light purple |
| Phenol | Light violet (24 hr.) | 0 | Light purple |
| Linalool | Brown-black | Light yellow-brown | Light yellow |
| *d* pinene | Yellow-brown | Light yellow-brown | Light pink |
| *d* limonene | Light brown | Light brown | Moderate orange |
| Guaiacol | Light yellow | Light yellow | Golden brown |
| Cadaverol | Light pink | Light yellow | Light yellow |
| Abietic acid | Yellow-red | Yellow-red | Yellow-red |
| Stearic acid | 0 | 0 | 0 |

Liebermann-Burchardt reagents.[5] A 2% and a saturated acetone solution were made with each compound examined. The Liebermann-Burchardt test was applied to a small portion of each solution. The color produced was compared with the blue-green color produced by cholesterol.

5. The Liebermann-Burchardt test consists of the addition of 5 drops of acetic anhydride to 1 cc. of an anhydrous solution of the material to be tested. One drop of concentrated sulfuric acid is added, and the color changes are observed over a period of 24 hours. When cholesterol is present a blue-green color develops almost immediately. (Koch, F. C., and Hanke, M. E.: Practical Methods in Biochemistry, Ed. 5, Baltimore, William Wood & Company, 1948.)

In order that a chemical may be examined by the Schultz test,[6] it must be adherent to a piece of tissue or similar material. Frozen sections of normal aorta that had been extracted with acetone for 24 hours were used. Several of these extracted sections were examined with scarlet red and the Schultz test to make sure that all compounds that might produce color with the latter test had been removed. A number of extracted sections were incubated in the solutions of each compound to be tested for at least 24 hours. A section from each solution was treated with acetic anhydride and concentrated sulfuric acid directly on a slide. When the color produced in these sections was similar to that produced by the compound in a test-tube reaction, it was assumed that the concentration of the compound in the tissue was sufficient for examination by the Schultz test. The Schultz test was then applied to these sections. Frozen sections of adrenal cortex, atherosclerotic aorta, and acetone-extracted aorta were used as controls.

## RESULTS

The intensity of the blue-green color produced in the Schultz test by sections of adrenal cortex was designated as 4+. The colors produced by the other materials were compared with this. When the reaction produced a blue-green color, it was designated as 1+ to 4+, depending on the intensity of the color produced.

## COMMENT

All the compounds tested except stearic acid produced a color reaction with the Liebermann-Burchardt test. Only linoleic acid, carotene, vitamin A, and ergosterol gave a color that could be confused with the blue-green color produced by cholesterol and cholesterol esters. Structurally these four compounds are not closely related to cholesterol. They all do have at least one double bond, however. Several of the sterols examined are closely related to cholesterol. It is interesting that none of these produced a color similar to cholesterol.

Of the group, only carotene (90% beta and 10% alpha) produced a color with the Schultz test that was indistinguishable from that produced by cholesterol and cholesterol esters. Carotenes are chemically closely related to vitamin A. They are found largely in plants and are usually converted to vitamin A in the higher animals.[7] Carotenes would, therefore, not ordinarily be important as a source of error in this histochemical test when used on animal tissue. Tissues taken from patients with carotenemia might possibly give a blue-green color in the absence of cholesterol. This should be tried when such tissue is available. The blue-purple color given by vitamin A can be distinguished from the blue-green color given by cholesterol.

The close correlation between the color produced by the Liebermann-Burchardt test in the test tube and the color produced by the Liebermann-Burchardt test on tissue sections with the various chemicals indicated that there was sufficient absorption of the chemicals in the sections of aorta to make them satisfactory for examination with the Schultz test.

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6. Frozen sections are used in the Schultz test. These sections are mordanted in a 2.5% iron and potassium sulfate solution of 37 C for three days. After being rinsed in distilled water, these sections are mounted on a slide and blotted dry. A drop of acetic acid-sulfuric acid mixture (1:1) is applied to the section. A blue-green color develops almost immediately when cholesterol is present. (Lillie, R. D.: Histopathologic Technic, Philadelphia, The Blakiston Company, 1948).

7. Harris, R. S., and Thimann, K. V.: Vitamins and Hormones, New York, Academic Press, Inc., 1943.

The purple color seen in the acetone-extracted aorta used as controls was very slight. It involved only a few fibers and did not interfere with interpretation of the test.

## SUMMARY

Twenty-four chemical compounds, including cholesterol and cholesterol esters, were examined by the Liebermann-Burchardt chemical test and the Schultz histochemical test. Some of the compounds had been found by previous investigators to produce a color reaction with the Liebermann-Burchardt test. Others were structurally related to cholesterol. Most of the compounds gave a color reaction with the Liebermann-Burchardt test. Only carotene, linoleic acid, vitamin A, and ergosterol produced a color that was similar to that produced by cholesterol and cholesterol esters. Carotene was the only compound which gave a reaction with the Schultz test that was indistinguishable from that produced by cholesterol and cholesterol esters. The Schultz histochemical test is, therefore, not entirely specific for cholesterol. It is, however, more specific than the Liebermann-Burchardt chemical test from which it was derived.

# EFFECT OF SEVERE ACUTE HEMORRHAGE ON KIDNEY OF RAT

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THAT RENAL function of man and animals is markedly reduced during hemorrhagic shock has been well established.[1] It also is well recognized that tubular degeneration in kidneys, sufficient to cause renal failure occasionally, occurs in man after the recovery from various forms of shock, including the shock associated with severe hemorrhage. In previous studies we have observed degeneration and necrosis of varied extent and severity in kidneys of dogs after prolonged hypotension produced by bleeding.[2] The purpose of the present investigation was to study (1) immediate changes in the renal vasculature and (2) the delayed pathologic changes in the kidneys of rats after sudden severe bleeding.

## METHODS

Albino rats weighing from 120 to 350 gm. were used. The animals were anesthetized with ether or pentobarbital sodium. The abdomen was opened so that the kidneys could be observed directly. In order that the physiologic effects of sudden severe hemorrhage might be reproduced,

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1. (*a*) Richards, D. W., Jr.: The Circulation in Traumatic Shock in Man, Bull. New York Acad. Med. **20**:363 (July) 1944. (*b*) Corcoran, A. C., and Page, I. H.: Effects of Hypotension Due to Hemorrhage and of Blood Transfusion on Renal Function in Dogs, J. Exper. Med. **78**:205 (Sept.) 1943. (*c*) Phillips, R. A.; Dole, V. P.; Hamilton, P. B.; Emerson, K., Jr.; Archibald, R. M., and Van Slyke, D. D.: Effects of Acute Hemorrhagic and Traumatic Shock on Renal Function of Dogs, Am. J. Physiol. **145**:314 (Jan.) 1946. (*d*) Selkurt, E. E.: Renal Blood Flow and Renal Clearance During Hemorrhagic Shock, ibid. **145**:699 (March) 1946. (*e*) Van Slyke, D. D.: Effects of Hemorrhage on the Kidney, Ann. New York Acad. Sc. **49**:593 (May 11) 1948. (*f*) Van Slyke, D. D.; Phillips, R. A.; Hamilton, P. B.; Archibald, R. M.; Dole, V. P., and Emerson, K., Jr.: Effect of Shock on the Kidney, Tr. A. Am. Physicians **58**:119, 1944. (*g*) Hamilton, P. B.; Hiller, A., and Van Slyke, D. D.: Renal Effects of Hemoglobin Infusions in Dogs in Hemorrhagic Shock, J. Exper. Med. **86**:477 (Dec. 1) 1947. (*h*) Corcoran, A. C., and Page, I. H.: Post-Traumatic Renal Injury: Summary of Experimental Observations, Arch. Surg. **51**:93 (Sept.) 1945. (*i*) Lauson, H. D.; Bradley, S. E., and Cournand, A.: The Renal Circulation in Shock, J. Clin. Invest. **23**:381 (May) 1944. (*j*) Doniach, I., and Walker, A. H. C.: Combined Anterior Pituitary Necrosis and Bilateral Cortical Necrosis of the Kidneys, Following Concealed Accidental Hemorrhage, J. Obst. & Gynaec. Brit. Emp. **53**:140 (April) 1946. (*k*) Fleming, J. F. R., and Bigelow, W. G.: Microscopic Observations of the Living Mammalian Kidney: The Effect of Crush Injuries, Shock, and Adrenalin on the Cortical Blood Flow, Surgery **30**:994 (Dec.) 1951.

2. Block, M. A.; Wakim, K. G.; Mann, F. C., and Bennett, W. A.: Renal Lesions and Function Following Prolonged Experimental Hypotension, Surgery **32**:551 (Sept.) 1952.

blood was withdrawn rapidly from the inferior vena cava by aspiration with a needle and syringe. The amount of blood removed was approximately 2.5 to 3.5% of the body weight, or 30 to 40% of the estimated total blood volume. In a few instances blood was withdrawn slowly for purposes of comparison. These will be mentioned later. Changes in the surfaces of the kidneys were observed directly. The abdomen then was closed in those animals which were to be used for later examinations. For study of the distribution of blood through the kidneys after the rapid severe hemorrhage in acute experiments, Higgins India ink (after centrifugation) diluted with an equal part of isotonic solution of sodium chloride was injected into the inferior vena cava of rats.

A total of 55 rats was used in the study, 18 for acute experiments and 37 for studies several days after rapid withdrawal of blood. Four rats died shortly after the hemorrhage.

Renal tissue for microscopic study was placed immediately in a 10% solution of formalin for fixation and was stained with hematoxylin and eosin.

## OBSERVATIONS

*Acute Effects of Sudden Severe Hemorrhage on Renal Vasculature.*—On sudden removal (during approximately 15 to 30 seconds) of blood from the rat, the kidneys first became pale and their surfaces severely blanched. The blanching of surfaces of the kidneys usually was much more marked than that of the other visceral organs. In 2 to 10 minutes after the hemorrhage the surface of the kidney gradually resumed a pink color. The surface occasionally was mottled for a few minutes.

India ink injected intravenously during the period of marked blanching appeared only in a few portions of the kidneys (Fig. 1). Occasionally ink was distributed throughout the cortex, but little or no ink was present in the medulla. However, when injections were made 20 to 30 minutes after withdrawal of blood, a uniform and complete distribution of ink was observed throughout the kidneys.

When the blood was returned to the animal at the time of extreme blanching of the kidneys, the kidneys rapidly regained their normal color, and India ink became uniformly distributed through the kidney after intravenous injection. When the blood was slowly removed from the animal, the pronounced blanching of the kidneys was not observed, even though the total volume of blood finally removed was the same as that when the blood was removed rapidly. These changes occurred in the denervated as well as in the intact kidney.

*Delayed Effects of Sudden Severe Hemorrhage on the Kidney.*—The kidneys of 16 rats were removed one to five days after the rapid withdrawal of blood. Grossly the kidneys usually appeared normal except for paleness. Some had a mottled appearance. The cortex of the kidneys of 11 of these rats contained areas of tubular degeneration and necrosis, varying from small focal lesions consisting of vacuolar degeneration to involvement by necrosis of nearly all cortical tubular tissue (Figs. 2 and 3). The degenerative changes were not restricted to any one segment of the tubule. Glomeruli remained normal in appearance. Large areas of normal-appearing tissue were nearly always present. The inflammatory reaction usually was minimal or absent. Scattered hyaline casts were present in the tubules, but pigmented casts were rarely seen. When India ink was injected intravenously immediately before the kidneys were removed for study, the ink was found to be distributed uniformly throughout the kidney.

The acute severe hemorrhage was preceded by three days of restricted intake of water in nine additional rats. The restriction of water was continued after the hemorrhage, and the kidneys were removed two days later. Two of the rats died

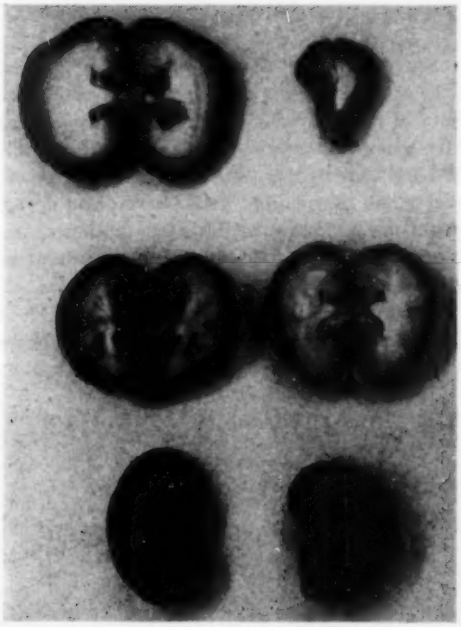

Fig. 1.—Patterns of intrarenal distribution of blood in kidneys of rats immediately after severe acute hemorrhage was induced.

The upper pair of kidneys is from a rat weighing 300 gm. from which 6.5 cc. of blood was rapidly removed. India ink was injected intravenously 15 minutes later. Ink is present only in the cortex and corticomedullary region of each kidney shown in sections.

The middle pair of kidneys is from a rat weighing 210 gm. from which 5.5 cc. of blood was rapidly removed. India ink was injected intravenously three minutes later. Ink is present only in certain areas of the cortex.

The lower pair of kidneys is from a rat weighing 220 gm. from which 5.5 cc. of blood was rapidly removed. India ink was injected intravenously 10 minutes later. Ink is present only in certain areas on the surface, producing a mottled appearance.

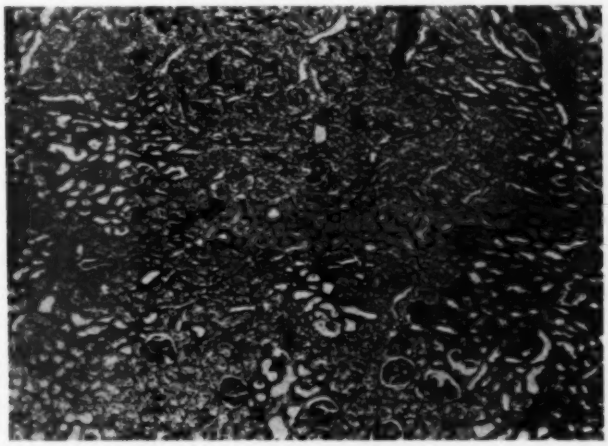

Fig. 2.—Area of extensive vacuolar degeneration and necrosis of tubular cells in the cortex of the kidney of a rat. Kidney was removed three days after acute severe hemorrhage. Hematoxylin and eosin; × 50.

24 hours after the hemorrhage. Tubular degeneration and necrosis were extensive throughout the cortices of the kidneys of six of the seven remaining rats (Fig. 4). The additional factor of restriction of water may have contributed to the increase in the severity of the lesions. Values for blood urea were determined on two of the rats, these being 96 and 649 mg. per 100 cc. respectively. After the same period of restriction of water only, small focal areas of vacuolar degeneration were frequently present in tubules of kidneys of rats, but the lesions were not nearly so severe or extensive as in kidneys of the rats in which hemorrhage was combined with restricted intake of water. Levels of blood urea after restriction of water of this duration were in the vicinity of 50 or 60 mg. per 100 cc.

After three days of restricted intake of water, blood was withdrawn rapidly from three additional rats, and after another two days of restricted intake of water the

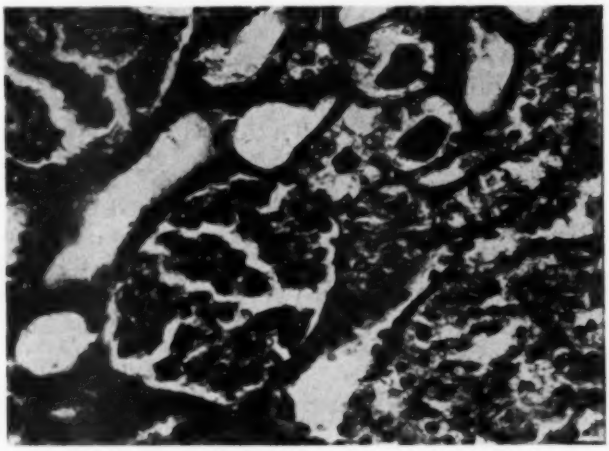

Fig. 3.—Vacuolar degeneration and necrosis of tubular cells and tubular casts in the area of the cortex of the rat kidney removed three days after acute severe hemorrhage (involved area is seen in the right half of the picture). The glomeruli appear normal microscopically. Hematoxylin and eosin; × 290.

rats were given water ad libitum. One of these rats died two days later, or four days after hemorrhage. The kidneys of the other two rats were removed three days after they again received water freely (five days after hemorrhage). The cortices of the kidneys of these rats contained large areas of tubular degeneration. The value for blood urea in one of these rats was determined at the time the kidneys were removed, and was 63 mg. per 100 cc.

Blood was withdrawn rapidly from three additional rats after they had been given a diet and water free of inorganic salts for one week, and after restricted intake of water for the three days preceding hemorrhage. One rat died 24 hours after hemorrhage. Widespread tubular degeneration and necrosis were present in the kidneys of the other two rats, examined three days after hemorrhage.

Blood was withdrawn rapidly from an additional three rats which had been receiving a diet and water free of inorganic salts for one week. The kidneys were

examined two days after the hemorrhage, and all were found to contain numerous areas of tubular degeneration and necrosis. The value for blood urea of one of these rats was 62 mg. per 100 cc. In other studies, the kidneys of rats receiving diet and water free of inorganic salts for one week did not contain microscopic abnormalities, although small areas of degenerative changes were present in kidneys of rats which had been receiving this diet for one to two months.

In three additional rats the sudden severe hemorrhage was followed immediately by electric stimulation of the nerves to the left kidney. The frequency and voltage used were those which had previously been found to produce optimal renal vasoconstriction.[3] The stimulation was continued for one hour. One rat died 24 hours later. The kidneys of the other two rats were removed three days after hemorrhage. Areas of degeneration in the tubules were found in both kidneys of each rat. The stimulation of the nerves to the left kidney did not appear to have influenced the changes which occurred. Continuous or interrupted electric stimulation of the renal nerves of one kidney for one to three hours in 10 additional rats in which hemorrhage was not induced failed to produce any histologic abnormality in either kidney removed two to five days after stimulation. Stimulation of the renal nerves in addition to water restriction for two to three days also failed to produce histologic abnormalities in other animals except for those changes attributable to severe restriction of water alone.

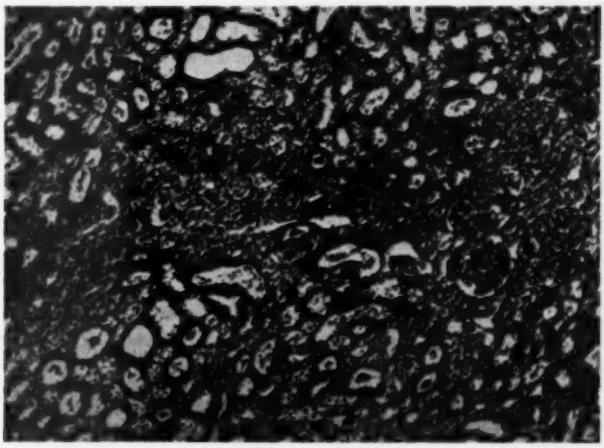

Fig. 4.—Scattered areas of vacuolar degeneration and necrosis of tubular cells in renal cortex of the rat. The kidney was removed two days after acute severe hemorrhage preceded by three days of water restriction. Hematoxylin and eosin; × 95.

Vacuolar degeneration was frequently present in parenchymal liver cells of rats, especially around the central veins, after the severe acute hemorrhage.

3. Block, M. A.; Wakim, K. G., and Mann, F. C.: Circulation Through Kidney During Stimulation of the Renal Nerves, Am. J. Physiol. **169**:659 (June) 1952.

COMMENT

Rapid and severe loss of blood in the rats is frequently followed by tubular degeneration of varying severity and extent in the renal cortices. The irregular and poor distribution of India ink in the kidneys after an intravenous injection within a few minutes after the severe bleeding is probably the result of renal vasoconstriction associated with a marked fall in blood pressure. However, the distribution of blood throughout the kidneys was usually uniform 20 to 30 minutes after the hemorrhage. Later studies of the intrarenal distribution of blood were not made in the acute experiments. Early vasoconstriction was more marked after abrupt than after slow hemorrhage, evidently as a consequence of a severer fall in blood pressure. Other investigators [4] have demonstrated that renal blood flow is decreased out of proportion to the fall in systemic arterial blood pressure in hemorrhagic shock. Fleming and Bigelow [1k] observed generalized constriction of arterioles and capillaries of the renal cortex of rabbits associated with low blood pressure produced by hemorrhage. However, blood did continue to flow through the vessels.

The injury to renal tissue was evidently not severe enough to be fatal in rats which survived the immediate effects of acute hemorrhage. Actually, the extent of renal degeneration usually was not great as observed microscopically. However, in a few instances death did occur after one to four days, and in a few animals the tubular necrosis in the kidney was severe and widespread. Evidently, before acute renal damage is fatal nearly all renal tissue must be severely damaged or rendered functionless. The study also suggests that the concomitant presence of dehydration may magnify the renal degeneration following severe hemorrhage. The extent of degenerative changes in the kidney varied greatly whether or not intake of water had been restricted. However, the severe loss of blood was the major factor involved in producing the degenerative changes, even though factors of water and electrolyte restriction were added. Other investigators have concluded that additional factors are important in increasing the renal damage associated with vasoconstrictive ischemia.[1b] Our studies agree with evidence obtained by Corcoran and Page [1b] indicating that the renal nerves are not an important factor in the loss of renal function associated with hemorrhage or shock. In fact, Corcoran and Page reported that the recovery of renal blood flow following relief of hemorrhagic shock by transfusion is slower in denervated kidneys than in intact kidneys.

The focal renal lesions in rats subjected only to severe water restriction could not be differentiated microscopically from those associated with acute severe loss of blood except that the lesions from the latter were much more extensive. Thus, the lesions related to severe restriction of water may be of ischemic origin. Moreover, renal ischemia may be an important factor in the experimental production of hemoglobinuric nephrosis by the combination of severe restriction of water and the intravascular injection of homologous hemoglobin.

The kidney of the rat is evidently more susceptible to the deleterious effects of hemorrhage than that of the dog. We rapidly removed 30 to 40% of the estimated blood volume from three dogs, but the kidneys showed no significant histologic changes when examined four to five days later. Several days of oliguria did follow

4. Richards,[1a] Selkurt,[1d] Lauson, Bradley, and Cournand.[11] Cournand, A.; Riley, R. L.; Bradley, S. E.; Breed, E. S.; Noble, R. P.; Lauson, H. D.; Gregersen, M. I., and Richards, D. W.: Studies of the Circulation in Clinical Shock, Surgery **13**:964 (June) 1943.

the withdrawal of blood, however. Reports of other investigators [5] also suggest that the kidney of the dog is relatively resistant to the injurious effects of acute hemorrhage and shock.

However, we have been able to produce degenerative changes in the kidney of the dog by abrupt and prolonged hypotension due to controlled bleeding.[2] The degeneration and necrosis in these kidneys were also usually most prominent in the cortex and varied in severity from small focal areas of involvement to bilateral cortical necrosis. In the study on dogs and in the study of rats, the degeneration was not limited to any one segment of the renal tubule, although the changes microscopically often appeared to be severest in the proximal convoluted tubules. Oliver and associates [6] observed areas of tubular degeneration occurring at random among nephrons and in any part of a nephron in cases of acute renal failure, and from dogs in which the renal artery had been temporarily occluded. These workers attribute the lesions to focal cortical ischemia.

## SUMMARY

Immediately after rapid withdrawal of a large quantity of blood from the albino rat, the blood flow frequently ceases through certain areas of the kidney. This is probably the consequence of renal vasoconstriction associated with a marked fall in arterial blood pressure. However, the distribution of blood throughout the kidney again becomes complete and uniform in 20 to 30 minutes.

Such an acute severe hemorrhage frequently results in tubular degeneration in the kidney of the rat. When the kidney was examined one to five days after the hemorrhage, the extent of injury varied from small focal areas of vacuolar degeneration in the tubules to widespread cortical necrosis. These degenerative changes, we believe, are a consequence of the ischemia. The lesions usually are not fatal and are not sufficiently extensive to produce significant renal insufficiency. The deleterious effects of acute severe hemorrhage in the rat are independent of renal innervation.

Severe restriction of intake of water preceding acute severe hemorrhage may increase renal damage.

5. Corcoran and Page,[1b] Netravisesh, V., and White, H. L.: Effects of Hemorrhage and Transfusion on Renal Circulation and Sodium Excretion in Dogs, Am. J. Physiol. **161**:442 (June) 1950. Smith, H. W.: The Kidney: Structure and Function in Health and Disease, New York, Oxford University Press, 1951. Van Slyke, D. D.: Effects of Shock on the Kidney, Ann. Int. Med. **28**:701 (April) 1948.

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# RIBONUCLEIC ACID IN CYTOPLASM OF LIVER CELLS

## Its Localization in Hyperplasia and Hepatoma Produced by 2-Acetylaminofluorene

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ALTHOUGH changes in the nucleic acid content of growing tissues have been reported from many laboratories, no consistent correlation has been established between rate of growth and alterations in nucleic acid concentration.[1] An increase of ribonucleic and desoxyribonucleic acid was observed in embryonic,[2] hyperplastic,[3] and malignant tissue,[4] through the use of histological techniques. Chemical analysis showed an increase in ribonucleic acid in growing or regenerating tissues but no significant alterations in nucleic acids in experimental and human tumors as compared with tissues of origin.[5]

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3. (*a*) Opie, E. L.: Mobilization of Basophile Substance (Ribonucleic Acid) in the Cytoplasm of Liver Cells with the Production of Tumors by Butter Yellow, J. Exper. Med. **84**:91-106, 1946. (*b*) Opie, E. L., and Lavin, G. I.: Localization of Ribonucleic Acid in the Cytoplasm of Liver Cells, ibid. **84**:107-112, 1946.

4. Stowell, R. E.: Nucleic Acid in Human Tumors, Cancer Res. **6**:426-435, 1946. Opie.[3a] Opie and Lavin.[3b]

5. (*a*) Novikoff, A. B., and Potter, V. R.: Biochemical Studies on Regenerating Liver, J. Biol. Chem. **173**:223-232, 1948; (*b*) Changes in Nucleic Acid Concentration During the Development of the Chick Embryo, ibid. **173**:233-238, 1948. (*c*) Johnson, R. M.; Albert, S., and Cohan, M. S.: Nucleic Acid Phosphorus in Normal Pregnancy Stimulated, and Cancerous Mammary Glands of Inbred Mice; abstracted, Cancer Res. **10**:227, 1950. (*d*) Schneider, W. C.: Intracellular Distribution of Enzymes: II. Distribution of Succinic Dehydrogenase, Cytochrome Oxidase, Adenosinetriphosphatase, and Phosphorus Compounds of Normal Rat Liver and in Rat Hepatomas, ibid. **6**:685-690, 1946. (*e*) Davidson. Davidson.[1a-c] Schneider.[1d]

The present study attempts to obtain information concerning the distribution and, within certain limits, the content of cytoplasmic ribonucleic acid in liver tissue, both in hyperplastic and in malignant cells in rats treated with the carcinogenic substance 2-acetylaminofluorene (N-2-fluorenylacetamide).

## MATERIAL AND METHODS

Wilson and co-workers [6] introduced the use of 2-acetylaminofluorene as a powerful carcinogenic agent. Skoryna and Webster [7] described the formation of hyperplastic nodules with or without accompanying cirrhosis and malignant tumors of the liver in rats following the administration of 2-acetylaminofluorene. It was found possible to produce nodular hyperplastic and neoplastic liver growth during or shortly after the period of 2-acetylaminofluorene administration.

Forty male and female albino rats (Wistar strain), weighing from 120 to 150 gm., were fed "standard" purina® meal and tap water ad libitum. For 36 to 42 consecutive weeks each animal received 5 mg. of 2-acetylaminofluorene daily. The animals were killed approximately 9 to 10 months after the beginning of the experiment.

The ribonuclease test, as introduced by Brachet [8] and surveyed by Stowell and Zorzoli,[9] was used in order to determine the localization and relative amount of ribonucleic acid in the cytoplasm. The cytoplasmic basophilia appearing with such dyes as the pyronine-methyl green stain or toluidine blue is ascribed to the presence of ribonucleic acid, and there is a good correlation between the basophilia of the cytoplasm and the ribonucleic acid content of the cell.[5] Incubation of tissues in solutions of the specific enzyme destroys its staining ability. Generally, ribonuclease destroys the basophilic inclusions in the cytoplasm of normal, as well as of hyperplastic and neoplastic, cells; it reduces the staining of the nucleoli, while leaving unchanged that of the nuclear meshwork.

Small pieces of liver tissue were fixed in Carnoy's or Bouin's fluid immediately after removal and embedded in paraffin. The deparaffinized sections were rinsed in water and then transferred into 0.1% solution of crystallized ribonuclease for one hour at room temperature, and afterwards into 0.05% ribonuclease solution at 40 C. for four hours. The control sections were incubated in the same way, distilled water being substituted for the enzyme solution. All sections were then stained with toluidine blue. No buffers were used.

## OBSERVATIONS

In normal liver tissue (Fig. 1) a regular structure, with polygonal cells arranged in cords, could be seen. Sections stained with toluidine blue showed varying inclusions of rod-like, filamentous, and granular appearance dispersed throughout the cytoplasm. These strongly basophilic cytoplasmic bodies disappeared after incubation with ribonuclease (Fig. 2). The staining of nucleoli became reduced.

In areas of focal hyperplasia (Fig. 3) enlarged cells of varying shapes were arranged in cords or in groups without any definite pattern. The cytoplasmic basophilia was more accentuated (Fig. 4) and appeared much more homogeneous than

6. Wilson, R. H.; DeEds, F., and Cox, A. J., Jr.: Toxicity and Carcinogenic Activity of 2-Acetylaminofluorene, Cancer Res. **1**:595-608, 1941.

7. Skoryna, S. C., and Webster, D. R.: Production of Cirrhosis and Liver Tumors in Male Rats Using High Dosages of 2-Acetylaminofluorene, Proc. Soc. Exper. Biol. & Med. **78**:62-67, 1951.

8. Brachet, J.: La détection histochimique des acides pentosenucléiques, Compt. rend. Soc. biol. **133**:88-90, 1940; footnote 2b.

9. Stowell, R. E., and Zorzoli, A.: Action of Ribonuclease on Fixed Tissues, Stain. Technol. **22**:51-61, 1947.

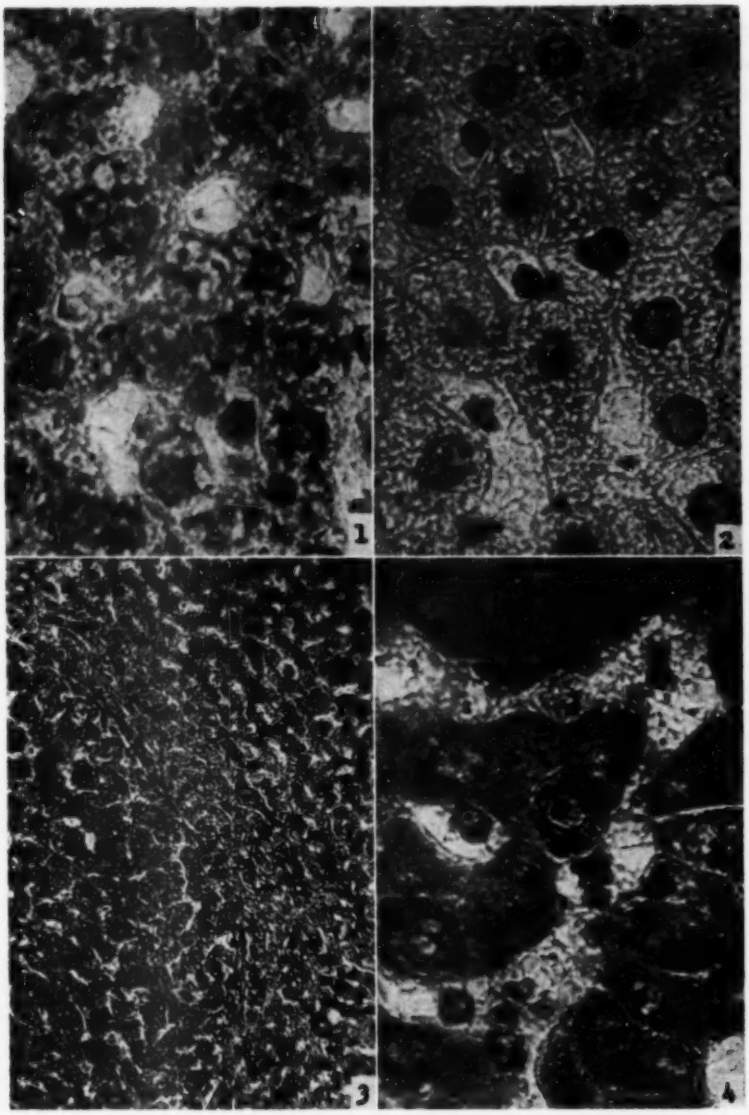

Fig. 1.—Normal liver tissue. Large basophilic bodies dispersed throughout the cytoplasm. Toluidine blue; × 1,000.

Fig. 2.—Section similar to that in Figure 1 treated with ribonuclease prior to staining. The cytoplasmic basophilia has disappeared. Toluidine blue; × 1,000.

Fig. 3.—Nodular hyperplasia (left lower corner). Hematoxylin and eosin; × 120.

Fig. 4.—Hyperplastic liver cells showing homogeneous appearance of basophilia. Toluidine blue; × 1,000.

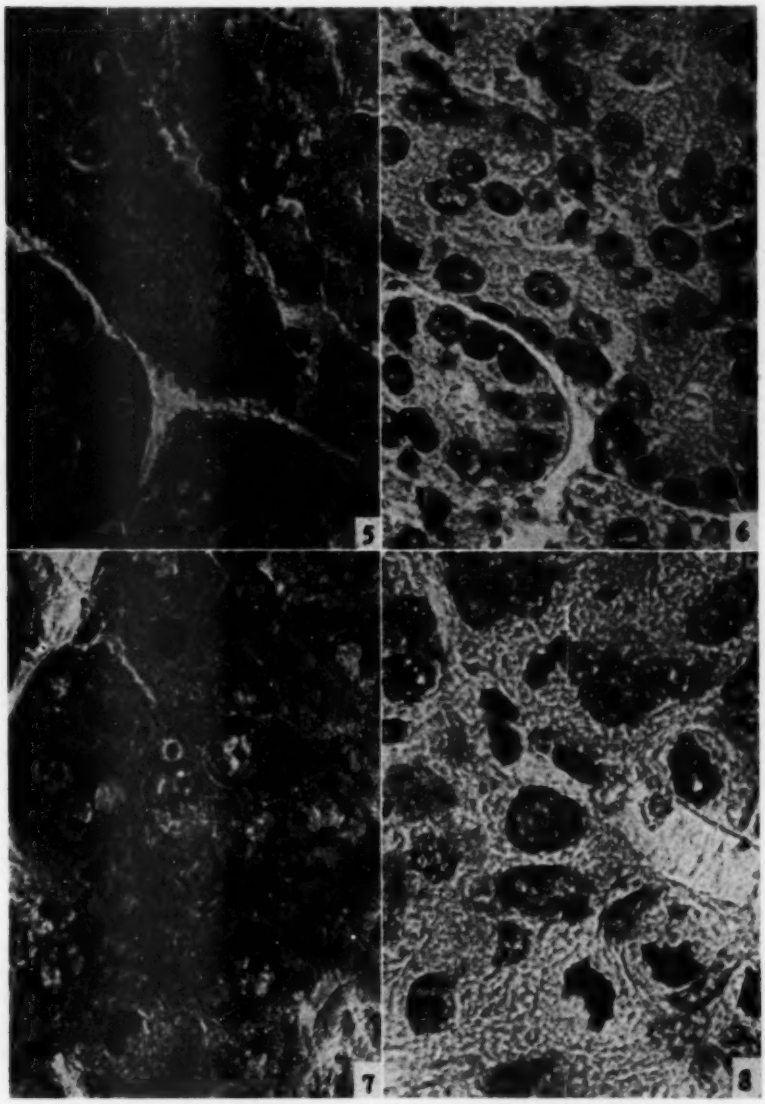

Fig. 5.—Hyperplastic liver tissue showing increased basophilia and fine granular distribution of basophilic bodies. Toluidine blue; × 1,000.

Fig. 6.—Section similar to that in Figure 5 incubated with ribonuclease prior to staining. The cytoplasmic basophilia has disappeared. Toluidine blue; × 1,000.

Fig. 7.—Dividing cells in hyperplastic areas showing fine granular distribution of the cytoplasmic basophilic material. Toluidine blue; × 1,000.

Fig. 8.—Section similar to that in Figure 7 treated with ribonuclease before staining, showing inhibition of basophilic staining of the cytoplasm. Toluidine blue; × 1,000.

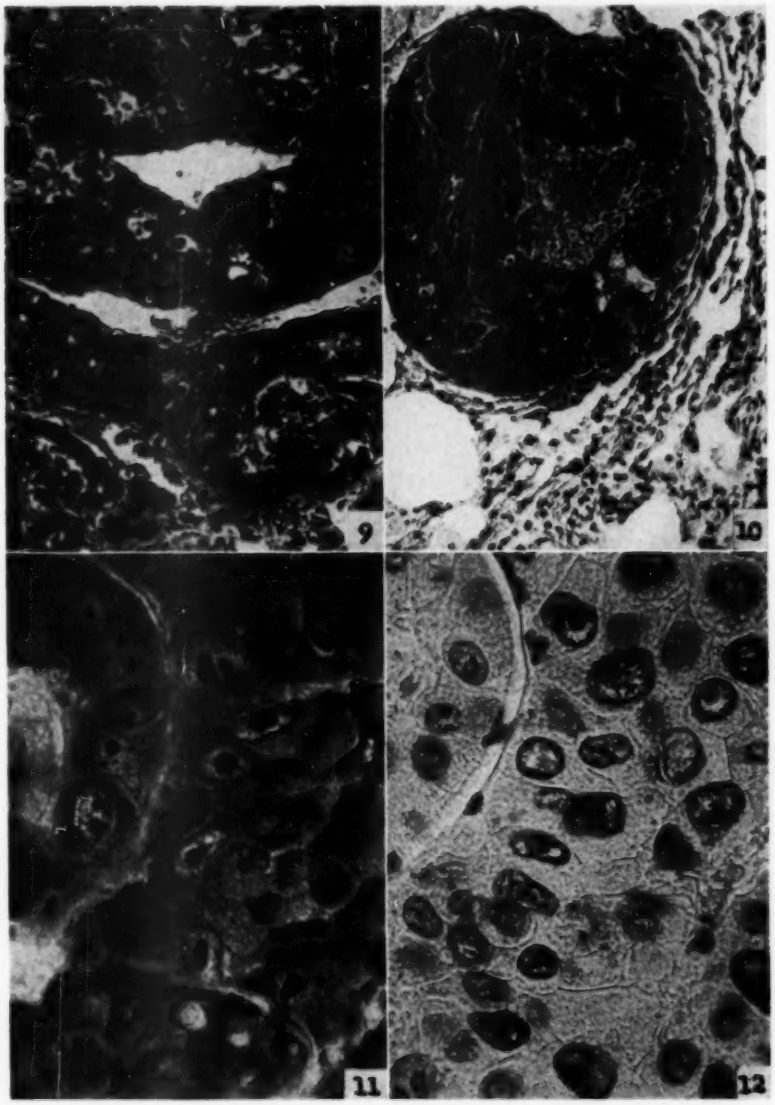


Fig. 9.—Malignant hepatoma. Hematoxylin and eosin; × 120.

Fig. 10.—Metastasis of the hepatoma in the lung. Hematoxylin and eosin; × 120.

Fig. 11.—Hepatoma cells showing increase in basophilia and homogeneous distribution of the cytoplasmic bodies. Toluidine blue; × 700.

Fig. 12.—Section similar to that in Figure 11 treated with ribonuclease before staining with toluidine blue. The basophilic material has disappeared. × 700.

that of adjacent resting cells. The cytoplasm was filled with uniformly distributed small basophilic inclusions in the form of fine granules or filaments. The enlarged nuclei contained one or more intact nucleoli.

Identical changes in distribution of cytoplasmic basophilia were observed in other areas of hyperplastic proliferation. The variously shaped, round and even pyramidal cells were arranged in a trabecular or columnar pattern around a lumen (Fig. 5). The strongly basophilic material was often accumulated in the periphery of the cell. In other regions the cells in mitosis were abundant (Fig. 7). The dividing cells did not present rod-like inclusions. Their cytoplasm was filled with finely granular, strongly basophilic particles. There seemed to be no increase in cytoplasmic basophilia in dividing as compared with resting liver cells. The corresponding sections incubated with ribonuclease showed no cytoplasmic basophilia (Figs. 6 and 8).

The malignant hepatoma presented varying degrees of differentiation, mostly of trabecular structure, with solid columns of cells and irregular lumens (Fig. 9). The cells were highly pleomorphic and presented more intensive basophilia than the cells of origin (Fig. 11). The granular basophilic bodies were diffusely dispersed throughout the cytoplasm of most cells, although accumulation of basophilic inclusions at the periphery was not uncommon. The nuclei showed many abnormalities, including hyperchromatism and giant, lobed, and muliple forms. Abnormal mitotic figures were abundant. After the incubation of the sections with ribonuclease, the cells lost their basophilia except for the chromatin material of the nucleus (Fig. 12).

Identical changes in the distribution and content of the basophilic material, cytoplasmic and nuclear, could be observed in the metastases of liver-cell carcinoma in the rat lung (Fig. 10).

The necrotic areas in primary tumors, as well as in metastases, in general failed to take basophilic staining. Correspondingly, treatment with ribonuclease was ineffective. Degenerated or disintegrating cells showed gradual loss of basophilia of the cytoplasm, depending on the state of necrobiosis. Their basophilia also disappeared after treatment with ribonuclease.

## COMMENT

In summarizing the histological evidence, it can be concluded, first, that there is an increase in the intensity of cytoplasmic basophilia in hyperplastic and in malignant cells as compared with normal liver tissue, indicating augmentation of ribonucleic acid content of the cytoplasm. Second, in the cytoplasm of normal liver cells the basophilic substances are coarsely distributed in rod-like and plump particles, whereas in hyperplastic and malignant cells fine, uniformly dispersed granules are seen. Third, in general, necrotic liver cells fail to take basophilic dyes, while degenerating cells show gradual reduction in their staining ability.

An increase in the intensity of cytoplasmic basophilia in hyperplastic and in malignant liver cells following the administration of dimethylaminoazobenzene (butter yellow) was observed by Opie.[3] With the use of a different carcinogenic agent, our observations indicate a similar increase in the ribonucleic acid content of the cytoplasm in both hyperplastic and malignant liver cells. Likewise, Novikoff and Potter [5a] found, by chemical analysis, an increase of over 50% in ribonucleic

acid concentration in compensatory hyperplastic tissue after partial hepatectomy. In experimental liver tumors as produced by dimethylaminoazobenzene and 2-acetylaminofluorene, however, no consistent alterations in ribonucleic acid concentration have been found by chemical methods. The ribonucleic acid content of the rat liver and that of the hepatoma are essentially the same, while the increase of desoxyribonucleic acid is related to the increase in the number of cells and nuclei in mitosis found in the tumor.[10]

Although the exact function of the basophilic structures of the cytoplasm is not definitely established, it is accepted that the structures show a great lability and are readily altered by any sort of injury to the cell. There are changes in the distribution and appearance of basophilic particles in connection with feeding, indicating alterations associated with nutrition and protein synthesis.[11] A single administration of carbon tetrachloride to mice causes gradual disappearance of the cytoplasmic basophilia, with a parallel decrease in ribonucleic acid concentration of liver tissue.[12] Opie [2a] described coarse basophilic particles in the cytoplasm of the normal rat liver. In the hyperplastic, as well as in the malignant, cells of hepatoma in rats, as produced by dimethylaminoazobenzene the cytoplasm showed fine granular stippling, distributed diffusely throughout. The basophilic inclusions have been identified as ribonucleic acid.

Since the changes in distribution and appearance of basophilic bodies in the cytoplasm reflect cellular activity, it seems that in this respect the hyperplastic cells are more closely related to malignant cells than to the normal liver cells. This is shown by the morphologic evidence in the distribution of basophilia, as well as by the relative increase in ribonucleic acid content, in both types of cells.

Necrotic cells show failure to take basophilic staining, while degenerating cells present a gradual reduction in their staining ability. According to Stowell and Lee,[12] the reduction of histochemical reaction for ribonucleic acid represents one of the earliest evidences of impending necrosis. Drochmans [13] observed no alterations in shape but noted the disappearance of cytoplasmic basophilia, with corresponding decrease in ribonucleic acid concentration, in ischemic liver tissue, as determined by chemical methods. This would illustrate that the ribonucleic acid content in tumors as determined by chemical analysis largely depends on the number of necrotic and degenerating cells present in the sample studied.[12] The varying amounts of connective and vascular tissue, inflammatory cells, and other types of tissue also influence chemical results, which usually provide an average value for different tissues. It is probable that the interference of such factors is responsible for the inconsistency in the chemical results of ribonucleic-acid determination.

10. (*a*) Davidson and Leslie.[1b] (*b*) Schneider, footnotes 1*d* and 5*d*.

11. Davidson.[1] Deane, H. W.: The Basophilic Bodies in Hepatic Cells, Am. J. Anat. **78**:227-238, 1946. Noël, R.: Recherches histo-physiologiques sur la cellule hépatique des mammifères, Arch. d'anat. micr. **19**:1-158, 1923.

12. Stowell, R. E., and Lee, C. S.: Histochemical Studies of Mouse Liver After Single Feeding of Carbon Tetrachloride, Arch. Path. **50**:519-537, 1950.

13. Drochmans, P.: Effect of Ischæmia on Nucleoproteins in Liver Cells, Experientia **3**:421-423, 1947.

SUMMARY

The distribution of ribonucleic acid in the cytoplasm of normal, hyperplastic, and malignant liver cells in rats fed with 2-acetylaminofluorene (N-2-fluorenylacetamide) was investigated by means of the ribonuclease-toluidine blue technique.

Normal liver cells show a coarse distribution of basophilic bodies, while fine granular structures dispersed uniformly throughout the cytoplasm are characteristic of hyperplastic and malignant cells. The latter cells show increased cytoplasmic basophilia, indicating augmentation in ribonucleic acid content. These differences seem to reflect different stages of functional activity.

A decrease or disappearance of basophilia is found in degenerating and necrotic cells. This result is discussed with respect to the discrepancies as obtained in chemical determinations of ribonucleic acid in malignant tissue.

# Case Reports

## DISSECTING INTRAMURAL HEMORRHAGE OF ANTERIOR DESCENDING BRANCH OF LEFT CORONARY ARTERY

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AND
W. J. CORZINE Jr., M.D.
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THERE are many discussions of isolated, relatively small intramural hemorrhages. However, few cases of intramural dissecting hemorrhage of the coronary vessels have been previously reported,[1] and these hemorrhages were in so-called normal vessels. The rarity of reports of dissecting hemorrhage and of the underlying pathologic process warrants the report of this case.

### REPORT OF CASE

K. F., a 39-year-old para 4-0-0-4[1a] white woman, was first seen in the obstetrical clinic at the Lutheran Hospital of Maryland on June 30, 1950, in her fourth month of pregnancy, with the chief complaint of fatigue. At that time her weight was 194½ lb. (88.3 kg.) and her blood pressure 122/72. On physical examination, no abnormality was noted except obesity. She was followed in the obstetrical clinic, but despite dietary efforts she gained 26½ lb. (12 kg.). On Nov. 16, a blood pressure of 140/80 was recorded; this was the highest reading obtained. Her past history was not significant. She had had four previous pregnancies, all of which were uncomplicated. No history could be obtained of significant past illnesses, and she denied having any cardiac symptoms. On Dec. 19, she was admitted to Lutheran Hospital in labor. She was given 100 mg. meperidine hydrochloride (demerol®) and 1/100 gm. (0.06 mg.) of scopolamine hydrochloride for analgesia. Three hours later the cervix was fully dilated, and, under nitrous oxide-oxygen anesthesia, she spontaneously and uneventfully was delivered of a full-term living female child. Blood pressure readings during labor and delivery varied from 130/70 to 120/80. Six days later, on Dec. 24, she was discharged, in good condition, after an afebrile, uneventful postpartum hospital course. On the 14th postpartum day, at about 10 a. m., she suddenly complained to her husband of moderately severe substernal pain, which occurred spontaneously, without exertion, and radiated to the base of the left scapula and the left shoulder. The pain was accompanied by mild dyspnea and cyanosis. Two hours later, she had another, severer attack of substernal pain with shortness of breath. Dyspnea and cyanosis were pronounced in the second attack and she lost consciousness. An ambulance was summoned, but she was dead on arrival at Lutheran Hospital, 2¼ hours after the initial onset of chest pain.

*Autopsy.*—The most striking gross alteration was an extensive dissecting hemorrhage of the anterior descending branches of the left coronary artery. The hemorrhage appeared to have originated in an atheromatous plaque located just distal to the ostium of the left coronary artery. From this location, the hemorrhage ruptured through the media and dissected beneath the adventitia, following along the main trunk of the artery and involving also the major

Assistant Medical Examiner, State of Maryland, Associate in Legal Medicine, University of Maryland School of Medicine (Dr. Lovitt), and Resident in Obstetrics, Lutheran Hospital of Maryland (Dr. Corzine).

1. Packard, M., and Wechsler, H. F.: Aneurysm of the Coronary Arteries, Arch. Int. Med. **43**:1, 1929.

1a. The formula 4-0-0-4 indicates the number of full-term infants delivered, the number of premature infants delivered, the number of abortions, and the number of children now alive (Greenhill, J. P.: Obstetrics in General Practice, Chicago, Year Book Publishers, 1948).

branches of the vessel. Even some of the small terminal branches about the apex of the heart were involved in the dissection. This massive dissecting hemorrhage elevated the intima and media, thus producing occlusion of the vessel. In the terminal branches the hemorrhage appeared to be entirely confined to the adventitial zone, whereas in the proximal areas the hemorrhage involved the medial layer and produced rupture of the muscle fibers. Grossly, there were only minimal zones of alteration of the wall of the left and right coronary vessels by atheromatous change. Autopsy showed no additional significant gross alterations other than obesity and a well-involuted postpartum uterus. The heart weighed 349 gm., and there was passive congestion of the liver (weight, 2,000 gm.). The right kidney weighed 150 gm.; the left kidney weighed 140 gm. and showed no gross abnormalities. Detailed examination of the lungs, gastrointestinal tract, brain, and endocrine glands showed no gross pathologic changes.

*Microscopic Examination.*—Serial sections of the coronary arteries showed a massive hemorrhage elevating the intima and occluding the lumen near the mouth of the left coronary artery. This hemorrhage produced a rupture of the subintimal

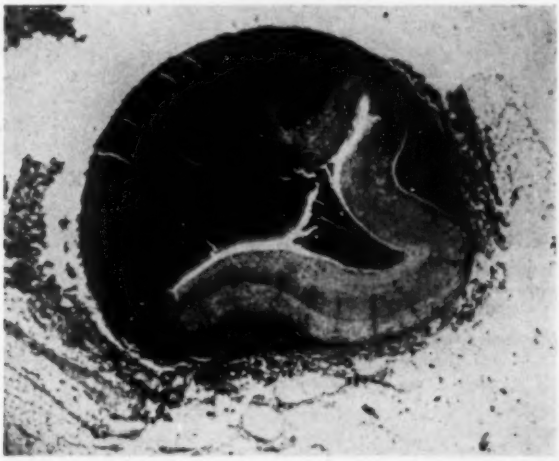

Fig. 1.—Section of left coronary artery near the ostium, showing extensive dissection by hemorrhage. Note the thin, intact intima separating the hemorrhage from the lumen and the rupture of the media. Hematoxylin and eosin stain following stain for elastic tissue; × 20.

tissues and the media and dissected around the vessel beneath the adventitia. At no place along the entire length of the main trunk of the left anterior descending artery did the dissecting hemorrhage communicate with the lumen. In all areas, there was at least a thin sheet of intimal and elastic tissue separating the hemorrhage from the lumen. Throughout the sections of coronary arteries, there were irregular thickening of the intima and alteration in the muscle cells of the media. These alterations consisted of an irregular myxomatous replacement of the individual muscle cells and groups of cells. Many of the adjacent muscle cells showed a homogeneous cytoplasm, with loss of fibrillae. Other cells showed granular degeneration of the cytoplasm and separation from the adjacent cells by large foamy macrophages. These atheromatous changes were apparent in both the right and the left coronary artery, as well as in the smaller arteries throughout the rest of the tissues. Throughout the sections near the mouth of the left coronary artery and

within the walls of both the right and the left coronary artery, there were numerous thin-walled vascular channels. In the pericardial fat about the adventitia of the left coronary artery there was a diffuse acute inflammatory response to the hemorrhage, manifested by polymorphonuclear leucocytes, with only an occasional lymphocyte.

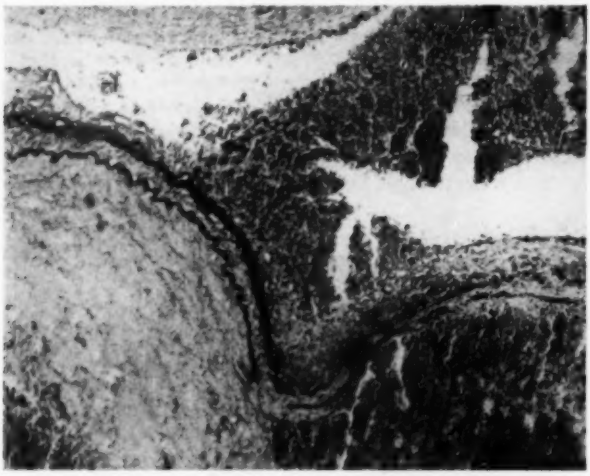

Fig. 2.—Section of left coronary artery. Note the irregular fragmentation of elastic fibers within the intima, as well as the subintimal fibrosis. Hematoxylin and eosin stain following stain for elastic tissue; × 140.

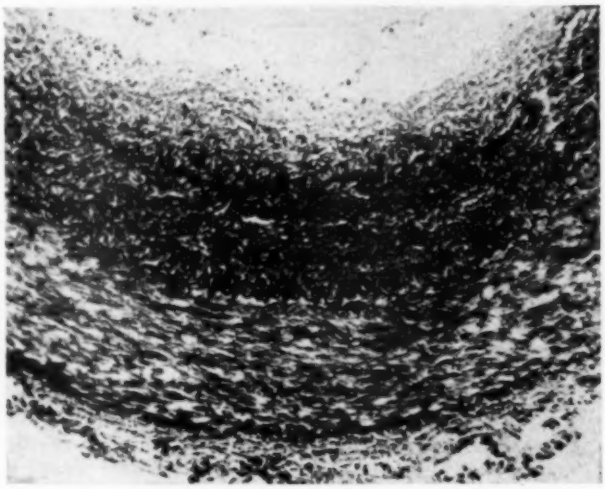

Fig. 3.—Section of right coronary artery showing extensive vacuolation of the media, irregular fibrosis, and fragmentation of elastic tissue throughout the intima. Note also the absence of adventitial inflammation. Hematoxylin and eosin stain following stain for elastic tissue; × 140.

This change was not present in the adventitia of the right coronary vessel and was thus considered to be a secondary reaction to the hemorrhage. Aside from the above changes, no significant alterations, either gross or microscopic, were noted throughout the remainder of the tissues.

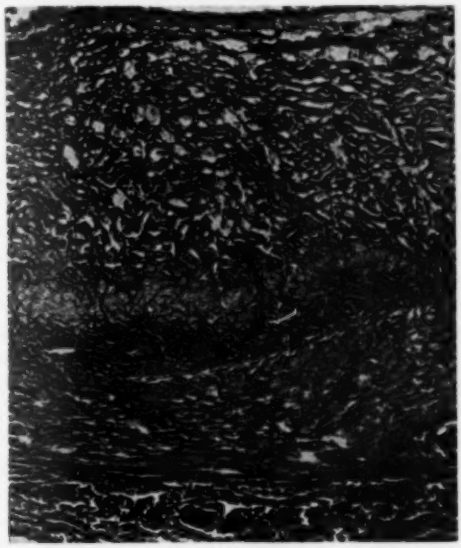

Fig. 4.—Section of the left coronary artery showing extensive atheromatous alterations of the intima. Hematoxylin and eosin stain; × 200.

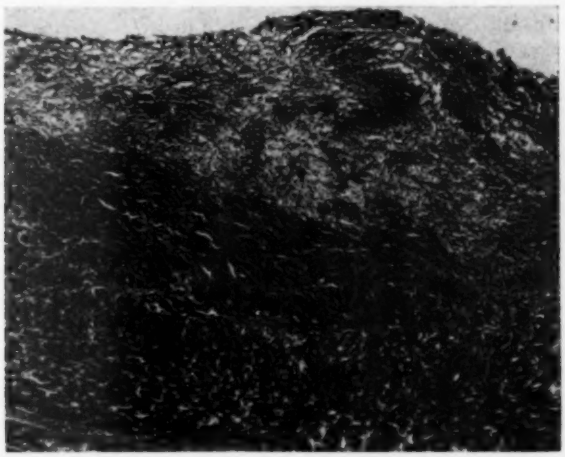

Fig. 5.—Section of the left coronary artery showing extensive atheromatous alterations of the intima. Hematoxylin and eosin stain; × 200.

## COMMENT

Many observers have noted isolated intramural hemorrhages in the walls of coronary arteries and have postulated that these hemorrhages arose from the vascularization of atheromatous plaques.[2] Winternitz and associates[2a] showed by an India-ink injection and clearing technique that some of the intramural vessels arose from the lumen of the artery. These vessels are thin-walled structures, containing in most instances only an endothelial lining and very little supporting wall, either of an intrinsic or an extrinsic nature. Consequently, these vessels are subject to rupture in conditions under which normal vessels would not be affected. Uehlinger[3] recorded two cases of dissecting aneurysms of the coronary artery in which the findings were similar to those noted in the present case. In each of these previous cases the hemorrhage followed strenuous exertion. Paterson[4] also believed that such intramural hemorrhages might be due to a sudden elevation of blood pressure during exertion. Since some of these thin-walled vessels arise from the lumen of the coronary arteries, an elevation in blood pressure during exertion would also produce an elevation of pressure in the thin-walled intramural vessels. After an intramural hemorrhage is produced, dissection could easily take place, especially in vessels with medial alterations such as those exhibited in our case. When the hemorrhage has broken into the adventitial zone, dissection can occur rapidly, with massive involvement of all the major branches. English and Willius[5] found that the most numerous and largest hemorrhagic lesions occurred in areas exhibiting large numbers of lipid-containing cells. The hemorrhage in the present case apparently arose near the ostium of the left coronary artery in a plaque which showed numerous lipid-filled cells, in addition to the intimal thickening.

The exact relationship of exertion to the features of this case is difficult to evaluate, as the only history of exertion is that which occurred during labor, 14 days prior to the patient's death. Possibly there is an interval between the initial hemorrhage and the dissection. In the other reported cases the delayed dissection of the hemorrhage was considered to be a part of the clinical picture. Additional cases of this peculiar condition need to be studied in order that the sequence of the pathologic alterations may be accurately determined.

## SUMMARY

A case of dissecting hemorrhage with occlusion of the left anterior descending coronary artery is presented. The hemorrhage was due to rupture of intramural vessels in an area of vascularization and atheromatous change. In addition, there were characteristic alterations of medial necrosis which allowed the hemorrhage to dissect throughout the length of the vessel and its major branches.

2. Horn, H., and Finkelstein, L. E.: Arteriosclerosis of the Coronary Arteries and Mechanism of Their Occlusion, Am. Heart J. **19**:655, 1940.

2a. Winternitz, M. C.; Thomas, R. M., and LeCompte, P. M.: The Biology of Arteriosclerosis, Baltimore, Charles C Thomas, Publisher, 1938.

3. Uehlinger, E.: Das spontane intramurale Hämatom und Aneurysma dissecans der normalen Coronararterie, Schweiz. med. Wchnschr. **77**:608, 1947.

4. Paterson, J. C.: Capillary Rupture with Intimal Hemorrhage as a Causative Factor in Coronary Thrombosis, Arch. Path. **25**:474, 1938.

5. English, J. P., and Willius, F. A.: Hemorrhagic Lesions of the Coronary Arteries, Arch. Int. Med. **71**:594, 1943.

# DIFFUSE GLOMERULAR NEPHRITIS AND LIPID NEPHROSIS

## Correlation of Clinical, Morphological, and Experimental Observations

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CERTAIN basic questions usually occur to the student of glomerular nephritis and lipid nephrosis. Is there only one diffuse glomerular nephritis, as believed by most investigators, or are there several kinds, as suggested by others? Is lipid nephrosis "a single distinct disease entity which is not preceded by unrecognized acute glomerular nephritis," [1] or is it "in reality a definite variety of subacute or chronic glomerulonephritis?" [2] "Why does blood escape into the urine in abundance in some cases [of glomerular nephritis] and not at all in others? What is there about glomerulitis that causes the urine in some instances to boil solid with albumin while only slight loss of albumin occurs in others? What mechanisms cause certain kinds of glomerular nephritis to become relentlessly progressive while others are completely resolved?" [2] What is lipid nephrosis, "why did the child develop it, and could it have been prevented? Will the child recover, and, if so, when, and will recovery be complete?" [1] Clinical and pathological studies have failed to answer these questions; clinicopathological correlation has been discordant, as emphasized by most modern writers.

Since the turn of the century investigators have attempted to produce experimental diseases that would throw light on the various problems pertaining to glomerular nephritis and lipid nephrosis. Renal diseases have been produced in rabbits by intravenous injection of antikidney serum or foreign protein and in rats by antikidney serum. The experimental diseases have been generally described as glomerular nephritis. The impact upon our concept of human nephritis and nephrosis by these experimental studies has been negligible.

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United States Public Health Service (Carolyn W. Forman).

Exhibited at the meeting of the American Association of Pathologists and Bacteriologists, and presented before the American Society for Experimental Pathology, Cleveland, April, 1951.

From the Philadelphia General Hospital, the University of Pennsylvania Graduate School of Medicine, and the Wyeth Institute of Applied Biochemistry, Philadelphia, and the Department of Pediatrics, The New York Hospital-Cornell Medical Center, New York.

1. Barnett, H. L.; Forman, C. W., and Lauson, H. D.: Nephrotic Syndrome in Children, in Advances in Pediatrics, edited by S. Z. Levine, and others, Chicago, Year Book Publishers, Inc., 1952.

2. Allen, A. C.: The Kidney, New York, Grune & Stratton, Inc., 1951.

Recently Heymann and Lund [3] recognized the similarity of the clinical pattern of antikidney serum disease in rats to the nephrotic syndrome in children. This observation led the pediatrician of our group (C. W. F.) to reinvestigate the rat disease. This was supplemented by a restudy of the nephritides caused by antikidney serum and foreign protein in rabbits. The experimental diseases were then compared with human nephritis and nephrosis. It will be shown that correlation of clinicopathological and experimental observations in renal disease is a method of solving many of the problems which in the past resisted elucidation.

In the microscopic study of renal lesions Ritter and Oleson's acid and periodate polysaccharide stain (ROPS)[4] was found to be illuminating. Excellent results with this stain were obtained also in decolorized hematoxylin and eosin stained formalin-fixed sections prepared 15 years previously (Fig. 4 *A*). Other stains that were found to be valuable are Beyer's trichrome stain (BTS),[5] Mullen and McCarter's fibrin stain (MMFS),[6] and Gomori's silver stain (GSS).[7]

## DIFFUSE GLOMERULAR NEPHRITIS AND LIPID NEPHROSIS IN MAN

### Previous Observations

Volhard [8] and Addis [9] believed that diffuse glomerular nephritis always begins with an "acute" or "initial" stage. Most patients recover promptly. Somewhat less than 20% die in an "acute, subacute, subchronic or entirely chronic" stage [8] or in an "initial, degenerative or terminal" stage.[9] Volhard and Addis agreed that the subchronic and entirely chronic, or degenerative and terminal, stages develop after a "lasting" or "latent" stage with little disturbance in renal function.

Volhard spoke of a subacute stage if death occurred weeks or months after onset. It was thought to be a continuation of the acute stage without development of a latent stage. Addis did not use the term "subacute." According to his terminology, the patient passes from the acute stage directly into the terminal stage. Volhard spoke of a subchronic stage if the patient died after months or years and of an entirely chronic stage if he lived for many years. The subchronic stage, according to Volhard, is frequently associated with the nephrotic syndrome; it is obviously the same as the degenerative stage of Addis.

Volhard pointed out that the subacute stage develops particularly in patients with a mild onset clinically. Similarly, Addis stated that the initial stage is difficult

3. (*a*) Heymann, W., and Lund, H. Z.: Lipemic Nephrosis in Rats, Science **108**:448, 1948; (*b*) Nephrotic Syndrome in Rats, Pediatrics **7**:691, 1951.

4. Ritter, H. B., and Oleson, J. J.: Combined Histochemical Staining of Acid Polysaccharides and 1,2 Glycol Groupings in Paraffin Sections of Rat Tissues, Am. J. Path. **26**:639, 1950.

5. Beyer, E. M.: Trichrome Stain for Astrocytes, Am. J. Clin. Path. Tech. Suppl. **4**:65, 1940.

6. Mullen, J. P., and McCarter, J. C.: Mordant Preparing Formaldehyde-Fixed Neuraxis Tissue for Phosphotungstic Acid Hematoxylin Staining, Am. J. Path. **17**:289, 1941.

7. Gomori, G.: Silver Impregnation of Reticulum in Paraffin Sections, Am. J. Path. **13**:993, 1937.

8. Volhard, F.: Die doppelseitigen hämatogenen Nierenerkrankungen, in von Bergmann, G., and Staehelin, R., editors, Handbuch der inneren Medizin, Berlin, Springer-Verlag, 1931, Vol. 6, Pt. 2, p. 1025.

9. (*a*) Addis, T., and Oliver, J.: Renal Lesion in Bright's Disease, New York, Paul B. Hoeber, Inc., 1931. (*b*) Addis, T.: Glomerular Nephritis, New York, The Macmillan Company, 1948.

to observe in patients whose lesions fail to heal. This was explained by both Volhard and Addis by the fact that these patients do not have gross hematuria or other remarkable symptoms and hence do not seek medical advice. Volhard stated that this happens in as many as 20% of cases of glomerular nephritis. He believed with Aschoff [10] that hematuria is absent, especially in those cases in which the glomerular alteration is so severe that the blood flow is completely obstructed.

Both Volhard [8] and Addis [9b] recognized a "genuine" lipid nephrosis. Addis did not distinguish at first between this malady and the degenerative stage of glomerular nephritis. Later he became convinced that the 20 patients whom he studied did not have glomerular nephritis.

Volhard [8] stated that lipid nephrosis differed from glomerular nephritis with the nephrotic syndrome by the fact that hypertension was always, and hematuria usually, absent. This observation is at variance with the statement of Blackman [11] that the character of the functional alterations of the kidneys is the same in glomerular nephritis and lipid nephrosis. Increased excretion of erythrocytes, rise in blood pressure, and elevation of nonprotein nitrogen occurred in both diseases. However, lipid nephrosis differed from glomerular nephritis by absence of gross hematuria and of continuous, progressive hypertension and retention of nonprotein nitrogen. The chief histological difference between the two diseases was believed to reside in the formation in nephritis of fibrin clots in the glomerular capsule, leading to destruction of the glomerulus and thus to renal insufficiency. Blackman concluded at first [11] that lipid nephrosis is indeed a distinct form of diffuse nephritis, but later he [12] felt that the two are essentially the same, the differences being in degree rather than in essence.

While Volhard and Addis believed that there is only one diffuse glomerular nephritis, Ellis [13] concluded that this is not a disease entity and that there are two different nephritides. His Type I nephritis was characterized by an abrupt onset (acute stage of Volhard, initial stage of Addis). Gross hematuria was present in 66% of these cases; preceding infection was demonstrable in 84%; recovery occurred in 82%. Of the patients who did not recover, 22% died during the acute stage, 22% weeks or months after a rapidly progressive course (subacute stage of Volhard), and 56% years after a slowly progressive course (entirely chronic stage of Volhard). Type II nephritis was characterized by its absence of an acute stage, its mortality rate of 95%, and its frequent association with the nephrotic syndrome. Of the patients who died, 63% died with uremia in less than one year to five years (subchronic stage of Volhard, degenerative stage of Addis).

Ellis pointed out that patients with lipid nephrosis who do not die early during the disease and in whom a chronic illness develops ultimately become indistinguishable from patients with his Type II nephritis. He also stated that histologically all gradations between nephrotic and nephritic changes were seen in early cases; some

10. Aschoff, L., cited by Volhard.[8]

11. Blackman, S. S.: Pneumococcal Lipoid Nephrosis and the Relation Between Nephrosis and Nephritis: I. Clinical and Anatomical Studies, Bull. Johns Hopkins Hosp. **55**:1, 1934.

12. Blackman, S. S.: On Pathogenesis of Lipoid Nephrosis and Progressive Glomerulonephritis, Bull. Johns Hopkins Hosp. **57**:70, 1935.

13. Ellis, A.: Natural History of Bright's Disease: Clinical, Histological and Experimental Observations: I. Nephritis, Lancet **1**:1, 1942; II. Essential Hypertension, ibid. **1**:34, 1942; III. The Vicious Circle in Chronic Bright's Disease, ibid. **1**:72, 1942.

patients who suffered from nephritis clinically showed nephrotic changes histologically. The glomerular lesions apparently consisted of thickening of the basement membrane and hyalinosis allegedly of the intercapillary space. Proliferative changes were present in some cases but absent in others. Ellis concluded that a separation of lipid nephrosis from Type II nephritis was not justified.

Findings of recent investigators [14] are in accord with the clinical observations of Blackman and Ellis. The results of clearance studies, however, are somewhat contradictory. Galán [14a] found that lipid nephrosis differs from glomerular nephritis in that the renal plasma flow and the glomerular filtration rate are normal or elevated and the tubular excretion and reabsorption capacity is at its maximum. As the ratio of tubular reabsorption to glomerular filtration was higher than in other diseases, he concluded that increased tubular reabsorption was a factor in the pathogenesis of lipid nephrosis. Metcoff and co-workers [14b] found that the renal plasma flow and the glomerular filtration rate are usually reduced, while the maximal tubular excretion capacity is usually normal or slightly reduced. When the disease persisted, all functions decreased progressively. Barnett and co-workers [1] observed that children with lipid nephrosis may have normal clearances and no hypertension throughout the disease and recover, while in others reduced clearances with or without hypertension may develop and they may either recover or die of renal insufficiency. But Galán distinguished between acute hemorrhagic nephritis, "nephronephritis" (mixed form), and uncomplicated nephrosis, while Metcoff and co-workers and Barnett and co-workers did not. The contradiction in the findings of these three groups may therefore be due to differences in material rather than in facts.

While the clinicians differ in their interpretations but generally agree in their observations, the pathologists are in no accord. Fahr's classic description [15] of diffuse glomerular nephritis and lipid nephrosis has been accepted by many but has been found inadequate by others. It is agreed that acute glomerular nephritis is characterized by infiltration of the tufts with polymorphonuclear leucocytes and by endothelial proliferation. The presence of thrombi within the capillaries during this stage, however, is believed by some to be rare, while others find it an essential part of the lesion. Crescents are generally looked upon as evidence of the subacute stage even though they are observed as early as the second day, and they may dominate the picture as much after 14 days as after months or years.[16]

14. (*a*) Galán, E.: Nephrosis in Children: Observations on 84 Patients; Clearance and Saturation Tests, Am. J. Dis. Child. **77**:328, 1949. (*b*) Metcoff, J.; Kelsey, W. M., and Janeway, C. A.: Nephrotic Syndrome in Children: Interpretation of Its Clinical, Biochemical, and Renal Hemodynamic Features as Variations of Single Type of Nephron Disease, J. Clin. Invest. **30**:471, 1951. (*c*) Barnett.[1]

15. Fahr, T.: Pathologische Anatomie des Morbus Brightii, in Henke, F., and Lubarsch, O., editors: Handbuch der speziellen pathologischen Anatomie und Histologie, Vol. 6: Harnorgane männliche Geschlechtsorgane, edited by T. Fahr, and others, Pt. 1: Niere, Berlin, Springer-Verlag, 1925.

16. (*a*) Koch, F.: Klinische und pathologisch-anatomische Untersuchungen zum Morbus Brightii, Ztschr. klin. Med. **115**:54, 1930. (*b*) Fahr, T.: Pathologische Anatomie des Morbus Brightii, in Henke, F., and Lubarsch, O., editors: Handbuch der speziellen pathologischen Anatomie und Histologie, Vol. 6: Harnorgane männliche Geschlechtsorgane, edited by H. Chiari, Pt. 2: Niere und ableitende Harnwege, Berlin, Springer-Verlag, 1934. (*c*) McManus, J. F. A.: Medical Diseases of the Kidney, Philadelphia, Lea and Febiger, 1950.

Fahr [15] stated that in cases of acute lipid nephrosis no changes were observed in most glomeruli but some showed swelling of the capillary wall and of the capsule with occasional adhesions; in older cases, these changes were more prominent and hyaline clumping of loops was present. He also noted infiltration of the glomeruli with cholesterol and occasional mild proliferative changes of epithelial cells. Fahr believed that these changes were sequels rather than the cause of the disease.

Patients who died during the "subchronic stage of glomerular nephritis" were found to show both endothelial proliferation and thickening of the basement membrane ("intracapillary glomerular nephritis") and epithelial and hyalinized crescents ("extracapillary glomerular nephritis"); the extracapillary changes outweighed the intracapillary alterations.[15]

Bell [17] distinguished (*A*) hemorrhagic glomerular nephritis without capillary obstruction, (*B*) diffuse proliferative glomerular nephritis (*a*) with intracapillary or endothelial proliferation and (*b*) with extracapillary proliferation or crescent formation, and (*C*) membranous glomerular nephritis.

Hemorrhagic nephritis (*A*) is characterized clinically by marked hematuria but no other symptoms of nephritis. No case of this nephritis was studied morphologically.

Diffuse proliferative glomerular nephritis (*B*) terminates in chronic nephritis and death in a high percentage of cases. Bell studied 51 cases of this nephritis of less than three months' duration. Only two showed gross hematuria. The microscopic picture is dominated by endothelial proliferation associated with "splitting of the basement membrane." A few epithelial crescents are found in the majority of cases. Occasionally crescents are so numerous that they overshadow the endothelial proliferation. When death occurs, after 3 to 12 months, wide variations are observed in the microscopic picture. Epithelial crescents are almost always present. Proliferation of endothelial cells is found in all cases, but often it is subordinate in importance to the epithelial crescents. Patchy focal thickening of the basement membrane was noted in only 13% of these subacute cases. The fate of the glomeruli in diffuse proliferative nephritis is hyalinization. The crescents are replaced by "collagenous fibers" derived from the epithelial cells.

Membranous glomerular nephritis (*C*) differs from proliferative glomerular nephritis by the absence of preceding infection and the presence of only a few red cells in the urine. The glomerular lesion in 67% of the cases consisted of thickening of the capillary basement membrane. In 16% of these it was focal and in 84% diffuse. Focal and diffuse endothelial proliferation occurred in 35% of these cases.

Bell apparently believed that acute hemorrhagic and diffuse proliferative glomerular nephritis are two different diseases, that the latter passes from an acute intracapillary stage through a "subacute" extracapillary stage into a chronic stage, and that lipid nephrosis is in reality a membranous glomerular nephritis. Bell, like Ellis, interpreted the thickening of the basement membrane as evidence of nephritis. He concluded that this disease may take either an azotemic or a hydropic course, depending upon the permeability of the injured membrane. In this view lipid nephrosis and glomerular nephritis are but different reactions to the same noxious agent.

17. Bell, E. T.: Renal Diseases, Philadelphia, Lea and Febiger, 1947.

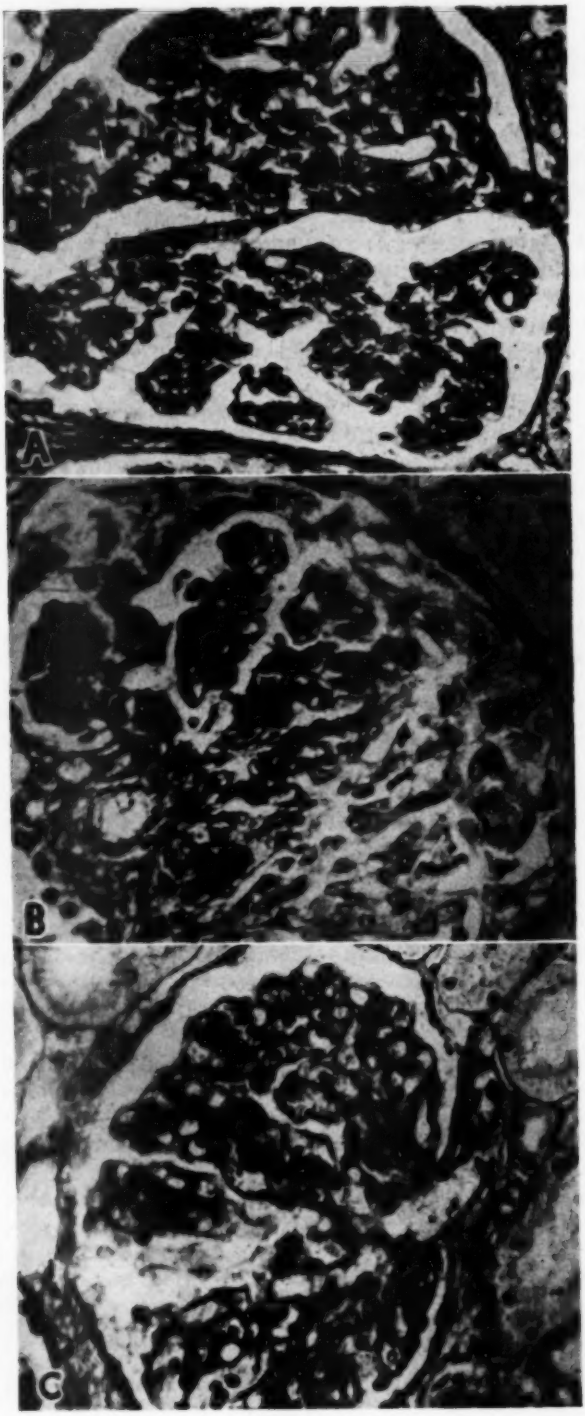


Figure 1

*(See legend on opposite page)*

McManus [16c] found crescent formation to be due, not to organization of hemorrhagic effusion, as suggested previously, but to "inflammatory" exudation, the capsular epithelial cells creeping over fibrin strands. The splitting of the basement membrane described by Bell was explained as new formation of reticular fibers by the proliferating endothelial cells. Patients who died with the nephrotic syndrome showed localized thickening of the basement membrane rather than new formation of fibers.

Allen,[2] like Bell, looked upon lipid nephrosis as a variety of glomerular nephritis. Like Bell, he found that it is characterized by specific histological alteration of the glomeruli, but he spoke of soft fibrinoid swelling of the capillary wall. In patients whose disease changes from lipid nephrosis to "chronic sclerosing glomerulonephritis," evidence of thickening of the capillary membrane can be detected among partially scarred glomeruli. Allen recognized a lobular variety of lipid nephrosis which is commonly confused with diabetic intercapillary sclerosis. The hyaline spheres in this variety are made up of silver fibrils; they are believed to be derived from the wall of the capillaries rather than from the intercapillary space.

## Present Observations

1. *Diffuse Glomerular Nephritis.*—Material: The glomerular changes in diffuse glomerular nephritis were studied in two children, 4 and 7 years old, with acute glomerular nephritis, dead on the fifth day of the disease, and in one adult, 69 years old, with subacute nephritis of unknown duration. Also examined were the kidneys of two children, 8 and 14 years old, who had no renal disease clinically but who died with active rheumatic fever.

Results: The two patients who died on the fifth day of the disease both showed the unadulterated picture of acute intracapillary glomerular nephritis (Fig. 1 *A*). The glomeruli were markedly enlarged. The capillaries contained few erythrocytes and no thrombi. The loops were infiltrated with varying numbers of polymorphonuclear leucocytes. There was marked increase in the number of endothelial cells. Sections stained with ROPS showed a delicate but distinctly stained basement membrane network with many cells in its meshes. Thickening of the membrane was inconspicuous. There was no exudate in the capsular spaces and no significant reaction of the epithelium.

The patient who died with subacute glomerular nephritis showed marked enlargement of the glomeruli, with considerable infiltration by polymorphonuclear leucocytes, but moderate proliferation of endothelium. Some loops contained thrombotic material, part of which stained yellowish with BTS and red with ROPS, indicating the presence of fibrin or fibrinoid; but some stained blue with BTS, suggesting collagenization. The outstanding change in this case consisted of marked crescent formation due to proliferation of both visceral and parietal epithelial cells in the great majority of the glomeruli (Fig. 1 *B*). The crescents included some fibrin or

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### Explanation of Fig. 1

Fig. 1.—*A*, intracapillary glomerular nephritis with marked endothelial proliferation and splitting of basement membrane in a 7-year-old child with 5 day history of acute glomerular nephritis. *B*, obliteration of tuft and crescent formation in a 69-year-old man with subacute glomerular nephritis. *C*, marked prominence of the intercapillary space in an 8-year-old child who died from rheumatic fever. ROPS; × 450.

fibrinoid and a good many fine fibers staining like collagen. The visceral basement membrane showed diffuse splitting and focal thickening in all tufts. The parietal basement membrane was irregularly thickened and split in places. Some loops of some glomeruli contained focal scars consisting of a dense network of mostly fine fibers staining like collagen and containing nuclei in their meshes. Other glomeruli showed all transitions, from a few patches of markedly thickened basement membrane to convolutions of shriveled masses of this membrane. All these changes occurred in the absence as well as in the presence of alteration of the capsular space. There were few completely hyalinized glomeruli. The tubules contained many hyaline casts but few erythrocytes. The interstitial tissue showed moderate fibrosis and round-cell infiltration.

The two patients who died of acute rheumatic fever did not show infiltration with polymorphonuclear leucocytes, endothelial proliferation, capsular exudate, crescents, or changes of the basement membrane, but both revealed a remarkable prominence of the intercapillary space (Fig. 1 *C*).

2. *Lipid Nephrosis.*—Material: This disease was studied in eight children 14 months to 7 years old. All had the nephrotic syndrome. Four died 18 days to 6 months after onset of the disease; the other four succumbed after 18 months to 3½ years.

Results: The first four patients showed no evidence of progressive renal failure. Interesting clinical highlights were (1) no hematuria; (2) no elevation of blood urea nitrogen in Cases 3 and 4, slight rise in Case 2 on two occasions, with reduction in urea clearance on one occasion, and acute elevation to 114 mg. per 100 ml. in Case 1 two days before death; (3) no rise in blood pressure in Case 3, and transient or terminal rises in the other cases, and (4) preceding acute tonsillitis in Case 1.

In all the other four patients progressive renal failure developed. Pertinent clinical points were (1) gross hematuria three days after diagnosis of the nephrotic syndrome in Case 5, occasional to many red blood cells in the urine of the other cases with an increase to 10,000,000 by Addis count one month prior to death in Case 8; (2) progressive rise in blood urea nitrogen and blood pressure noted one month before death in Case 6, six months before death in Case 7, and one year before death in Cases 5 and 8, and (3) preceding infection in Case 6.

As lipid nephrosis is looked upon widely as a clinical rather than a pathological entity, the pertinent clinical observations are given in detail.

Case 1.—A white girl, 19 months old, on Oct. 1, 1949, fell ill with tonsillitis, followed on the third day by puffiness around the eyes and on the sixth day by anasarca. In the following 10 days the patient gained 6 to 7 lbs. (2.7 to 3.2 kg.) in weight, and she became oliguric and lethargic. On admission to the hospital on Oct. 13 she was grossly edematous. Urinary protein was 4+, serum protein 4.0 gm., albumin 1.6 gm., cholesterol 1,392 mg., blood urea nitrogen 82 mg. per 100 ml.; the blood pressure was 90/60 mm. Four days later the blood urea nitrogen was 114 mg. per 100 ml. and the blood pressure 140/90 mm. She also had hyperpotassemia. The patient died on Oct. 19, 18 days after onset of the disease.

Case 2.—A white boy, 3½ years old, was known to be sensitive to dust, ragweed, and wool, by skin test. On Oct. 23, 1947, a few pimples and edema developed around his eyes, followed by anasarca and oliguria three days later. On Admission to the hospital on Oct. 29 he had edema, 2+. Urinary protein was 4+, serum protein 5.0 gm., albumin 2.0 gm., cholesterol 1,085 mg.,

blood urea nitrogen 24 mg., and hemoglobin 12 gm. per 100 ml.; the blood pressure was 110/60 mm. Urea clearance was 19.3% on Nov. 7, and the blood urea nitrogen level was 57 mg. per 100 ml. on Nov. 18. An upper respiratory infection developed on Nov. 28, followed by rise in diastolic blood pressure to 90 mm. After discharge from the hospital on Dec. 10 he was seen in the clinic on Dec. 18, when he had an eczematoid rash on the legs and arms and the blood pressure was 105/75 mm. Early in 1948 another upper respiratory infection developed and he gained 4 to 5 lb. (1.81 to 2.27 kg.) in weight and began to vomit and have diarrhea. On readmission on Jan. 10 he was acutely ill, with massive edema and hyperpotassemia. The serum protein was 3.9 gm. and blood urea nitrogen 44 mg. per 100 cc., and diastolic blood pressure 90 mm. He died approximately 20 hours after readmission, less than three months after onset of the disease.

Case 3.—Three months prior to admission, after a mild upper respiratory infection, generalized edema developed in a 7-year-old girl. Urinalysis at that time revealed protein and casts in her urine. She was treated with a high-protein and low-salt diet and intravenous injections of an unknown drug. One day before admission nausea and rapid respiration developed. On admission to the hospital, on July 24, 1937, she was a very sick child, who was markedly edematous and had extreme abdominal tenderness. She also had a profuse nasal discharge and a temperature of 105 F. Her hemoglobin level and red blood cell count were normal. Her white blood cell count was 5,000, with 70% polymorphonuclear leucocytes, showing a marked shift to the left. Urinalysis revealed protein (4+) and three casts per high power field. The urine contained white blood cells but no red blood cells. Her serum protein was 3.4 gm., albumin 1.5 gm., cholesterol 480 mg., blood urea nitrogen 19 mg., nonprotein nitrogen 38 mg., and uric acid 3.8 mg., per 100 ml. A chest roentgenogram revealed left pleural effusion. Blood and spinal fluid cultures yielded pneumococci, nonspecific as to type, in pure culture. Nasal cultures revealed pneumococci and staphylococci in small numbers. The child died on July 27, 1937, three months after onset of the disease.

Case 4.—A white girl, 2 years of age, five months prior to admission began to have puffiness about the eyes and swelling of the abdomen. After treatment by plasma transfusion and the Fox regimen (potassium acetate and sodium acetate) most of the edema disappeared. On admission to the hospital she had a temperature of 102, edema (4+), ascites, and erythema over her lower back. Serum protein was 3.7 gm., albumin 1.0 gm., blood urea nitrogen 10 to 15 mg. and hemoglobin 8.0 to 12.4 gm. per 100 ml., and blood pressure 118/90 to 150/120 mm. The urine contained rare hyaline casts. Its specific gravity was 1.022 to 1.030. She continued to gain weight, and an erysipeloid rash developed over the vulva and back and a wound infection at the site of an ankle cut-down. She died six weeks after admission, more than six months after onset of the disease.

Case 5.—In August, 1947, "pure nephrosis" with swelling of eyelids and proteinuria followed by gross hematuria developed in a 7-year-old white boy. His serum protein was 3.5 gm., albumin 1.3 gm. cholesterol 470 mg., and blood urea nitrogen 20 to 36 mg. per 100 ml. He did not respond to chickenpox infection and salt-poor albumin therapy. On admission to the hospital on March 4, 1948, urinary protein was 3+. The sediment contained many red blood cells. Serum protein was 3.4 gm., albumin 1.2 gm., cholesterol 467 mg., and blood urea nitrogen 23 to 33 mg. per 100 ml., and blood pressure 142/104 mm. Urea clearance was 38% on two occasions. After his discharge from the hospital he continued to be edematous. On his last admission, on Feb. 1, 1949, serum protein was 4.0 gm., albumin 2.3 gm., cholesterol 396 mg., and blood urea nitrogen 126 mg. per 100 ml., and blood pressure 140/122 mm. He died on Feb. 3, 1949, one year six months after onset of the disease.

Case 6.—A white boy, 14 months old, in February, 1948, had a rash three days following a rise in temperature to 104 F. This was followed by puffiness around the eyes on March 15 and a severe upper respiratory infection on March 30. On admission to the hospital on April 4, 1948, he had moderate generalized edema. His urine contained protein (3+) and many red blood cells. The serum protein was 4.7 gm., albumin 1.7 gm., cholesterol 658 mg., and blood urea nitrogen 10 mg. per 100 ml.; the blood pressure was 135/80 mm. After discharge from the hospital he had remitting edema. In March, 1949, he was operated on for obstructed umbilical

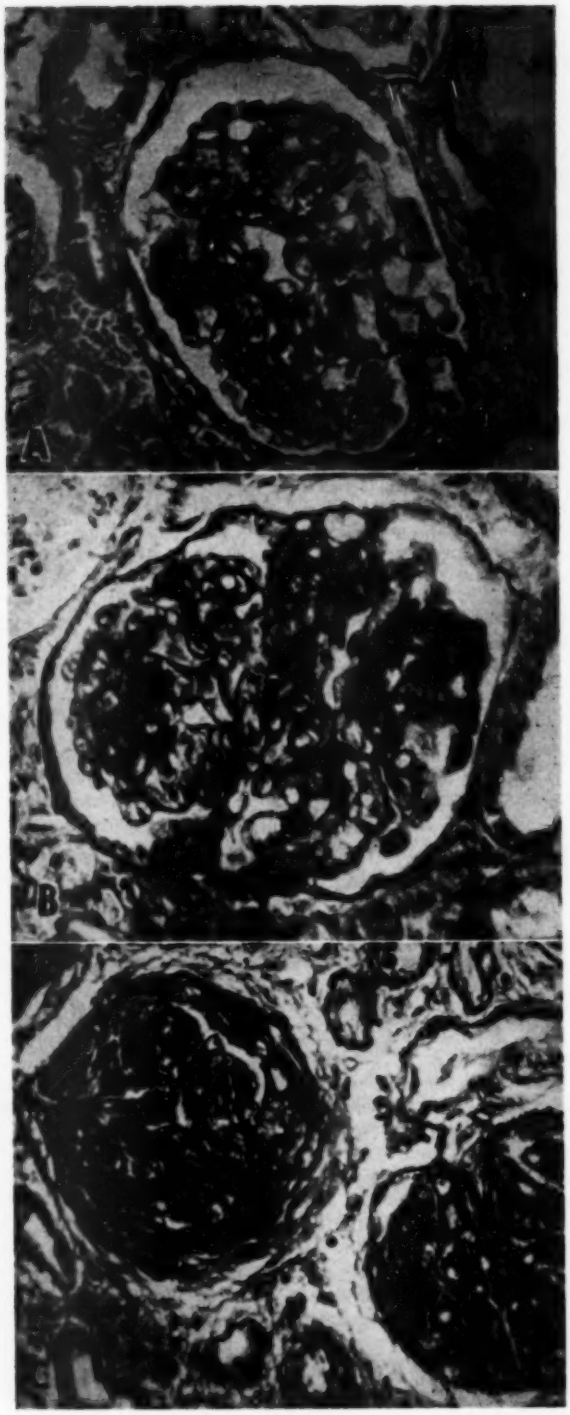


Figure 2

*(See legend on opposite page)*

hernia. On his third admission to the hospital, on Sept. 19, 1949, he had massive edema. The total protein was 3.5 gm., albumin 1.9 gm., cholesterol 753 mg. and blood urea nitrogen 61 mg. per 100 ml. The blood pressure was 160/110 mm. On his last admission, on Oct. 11, 1949, he had bronchopneumonia, edema (4+), and a blood pressure of 160/110 mm. He died a few hours after readmission, that is, one year eight months after onset of the disease.

CASE 7.—A white girl, 16 months old, in April, 1944, was noted to have edema around the eyes followed by intermittent anasarca and oliguria. In November, 1944, she had an upper respiratory infection with proteinuria. On admission to the hospital on July 2, 1945, she had edema (2+). Urinary protein was 3+, serum protein 4.5 gm., albumin 1.7 gm., cholesterol 574 mg., and blood urea nitrogen 14 mg. per 100 ml. On her second admission, on Sept. 16, 1945, she had edema (2+) and ascites. Urinary protein was 3+. There were occasional red blood cells in the urine. Serum protein was 4.3 gm., albumin 1.3 gm., cholesterol 722 mg., and blood urea nitrogen 9 mg. per 100 ml. On her third admission she had asthmatic bronchitis and ascites. Her cholesterol was 753 mg. per 100 ml., blood urea nitrogen 9.2 mg., and blood pressure 112/50 mm. During the following 12 months she had six hospitalizations. She was never free of edema. The blood urea nitrogen and blood pressure were found to be elevated first on Aug. 17, 1946, that is, two years four months after onset of the disease. Blood urea nitrogen at that time was 59.5 mg. per 100 ml. and blood pressure 138/90. Serum protein was 4.1 mg., albumin 1.7 mg., and cholesterol 1,246 mg. per 100 ml. On her last admission, shortly before death, she had edema (4+). Serum protein was 2.3 gm., albumin 1.5 gm., cholesterol 1,020 mg., and blood urea nitrogen 87 mg. per 100 ml. and the blood pressure was 145/108 mm. She died three years after onset of the disease.

CASE 8.—A white girl 5½ years old, on examination, on Dec. 26, 1944, after the development of nephrosis in her twin sister on Nov. 11, was found to have proteinuria (4+). Serum protein was 4.4 mg., albumin 3.1 mg., cholesterol 424 mg., and blood urea nitrogen 12 mg. per 100 ml., and the blood pressure was 70/44 mm. In the following year she had four hospitalizations. She had upper respiratory infections and remittent edema. Urinary protein was 4+, and she had red blood cells in her urine. Serum protein varied from 3.1 to 5.4 gm. and blood urea nitrogen from 9 to 34 mg. per 100 ml., and blood pressure varied from 104/58 to 125/26 mm. Urea clearance was 31.6%. During the second year of her illness she had eight hospitalizations. The blood urea nitrogen rose from normal in August, 1947 to 40 mg. per 100 ml. in March, 1948, and to 88 mg., in May, 1948. The blood pressure, which was normal until this time, rose to 160/96 mm. The red blood cells in the urine approached 10,000,000 by Addis count. She died on July 13, 1948, three years seven months after onset of the disease.

The morphological appearance of the kidneys of the first four cases was monotonously similar. The glomeruli were uniform in appearance. The tufts were of usual size with no increase in cellularity. The capillaries contained various amounts of blood. The blood content was lowest in the first patient, who died 18 days after onset of the disease, and in whom there was a marked rise in blood urea nitrogen and blood pressure before death. No significant changes of glomeruli were seen in sections stained with hematoxylin and eosin. Sections stained with BTS showed

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EXPLANATION OF FIG. 2

Fig. 2.—*A* (Case 3), thickening of basement membrane associated with marked increase in number and size of visceral epithelial cells in 7-year-old child with 3 month history of lipoid nephrosis. *B* (Case 3), thickening of basement membrane, focal sclerosis, and adhesion of a glomerulus in a 7-year-old child with 3 month history of the nephrotic syndrome. The child showed at no time hematuria, rise in blood pressure or evidence of renal impairment. *C* (Case 7), obliterated glomerulus with markedly thickened basement membrane surrounded by a hyalinized crescent staining blue instead of red with ROPS indicating that it may be related to hyaluronic acid. The child was 4½ years old and had a history of the nephrotic syndrome of 3 years' duration with progressive renal failure. ROPS; × 450.

some glomerular changes in some kidneys. However, sections stained with ROPS revealed alterations in the glomeruli of all cases, namely focal thickening and "splitting" of the basement membrane, thickening being more prominent than splitting (Fig. 2 *A*). In Case 3 there was marked thickening but hardly any splitting. In Case 1 there were also occasional hyaline masses in the capillaries. Focal scars of loops were very rare or absent in Cases 1, 2, and 3. In Case 4, however, definite focal scars were observed in approximately 10% of the glomeruli (Fig. 2 *B*). These were associated with rare adhesions (Fig. 2 *B*). The scars consisted of a dense network of irregularly thickened collagen fibers containing nuclei in their meshes but showing few patent lumina. The endothelial cells were not increased in number, but in some cases appeared to be enlarged and markedly infiltrated with fat. There was protein in some capsular spaces. The visceral epithelial cells were increased in number and size, especially in places of basement membrane alteration and scar formation (Figs. 2 *A* and *B*). The parietal epithelium showed similar changes. However, there were no crescents. The parietal basement membrane was not altered. Three children who died with evidence of infection showed swelling of the intercapillary space similar to the reaction observed in rabbits with nephritis produced by injection of foreign protein (p. 479). Most of the tubule lumens were wide and contained protein, many desquamated epithelial cells, and many hyaline casts; erythrocytes were absent in all cases. The epithelial lining of the proximal convolutions showed the usual infiltration with hyaline droplets and fat.

The kidneys of the second group of patients (Cases 5 to 8) were quite different from those of the first. The glomeruli varied greatly in appearance. Some were markedly enlarged and appeared capable of functioning; others were completely obliterated and apparently without function. There were varying numbers of adhesions and well-developed epithelial crescents. The tufts showed an increase in endothelial cells in one case (Case 6). Their basement membrane was markedly altered in all cases. In Case 6 the membrane was comparatively delicate but split with many endothelial cells in its meshes. The other cases showed heavy thickening and fusion of the basement membrane with comparatively little splitting. Many tufts showed marked focal to diffuse fibrosis and hyalinosis. The glomeruli which were completely obliterated consisted of a core of conglomerated, shriveled, very thick basement membrane. In Case 5 the core was surrounded by thick crescents of laminated hyaline material staining blue with ROPS (Fig. 2 *C*). In Cases 6 and 7 this was inconspicuous. The original parietal basement membrane was irregularly thickened and disintegrated in places. The tubules contained numerous erythrocytes and hyaline casts. The epithelium of the proximal convolutions showed hyaline droplets. The interstitial tissue was markedly fibrotic and infiltrated with round cells. Fat stains were not available in these four cases.

### ANTIKIDNEY SERUM NEPHRITIS IN RABBITS

#### Previous Observations

The renal disease caused by antikidney serum in rabbits has been uniformly found to resemble diffuse glomerular nephritis in man.[18] Masugi[18a] noted that the

18. (*a*) Masugi, M.: Über die experimentelle Glomerulonephritis durch das spezifische Antinierenserum, Beitr. path. Anat. **92**:429, 1933-1934. (*b*) Hemprich, R.: Zur Frage der experimentellen Glomerulonephritis, Ztschr. ges. exper. Med. **95**:304, 1935. (*c*) Weiss, A.: Weitere Beiträge zur Frage der experimentellen Glomerulonephritis, Beitr. path. Anat. **96**:111,

*(Footnote continued on next page)*

disease begins after a latent period of approximately one week following the injection of serum. This was later interpreted to mean that this nephritis is due not to a simple reaction between the nephrotoxin (antibody) and the glomerulus (antigen) but to an associated Arthus reaction to the foreign serum in which the nephrotoxin is contained. Antibody against this serum is produced by the recipient.[19]

The clinical findings in this experimental nephritis were last summarized by Kay.[18h] There are oliguria, proteinuria, cylinduria, and moderate edema. Gross hematuria was noted repeatedly.[18a-e] Rises in blood urea nitrogen and in blood pressure were likewise observed; they were found to be absent in mild cases.[20] Depression in the filtration of cyanhydrin with little or no depression in the secretion of azofuchsin I was also found.[18f] There was no demonstrable change in the renal circulation during the acute phase of the disease.[18g]

The microscopic changes in this disease were last reviewed by one of us (W. E. E.).[18f] It was pointed out that there are two distinct alterations, intracapillary and extracapillary, closely resembling the changes in man. Significant proliferation within the glomerular tuft and exudation into the capsular space were both observed first eight days following the injection of serum. The proliferation affected all glomeruli. Exudation varied considerably. The number of glomeruli affected by exudation ranged from 1 to 92%. Crescents developed only in glomeruli in which exudation had occurred. Both exudation and proliferation were preceded by hyperemia and infiltration of the capillary loops with polymorphonuclear leucocytes. The latter occurred as early as the first day following injection.

Coagulation of protein and fibrin thrombosis within the capillaries and imbibition with this material of the capillary wall during the acute phase of this nephritis were noted by Masugi.[18a] When healing occurred, coagulated protein and fibrin disintegrated into granules or was washed out. In the absence of healing, sclerosis ensued. In chronic cases many glomeruli were found to be obliterated by sclerosis and hyalinosis.[21]

### Present Observations

*Materials and Methods.*—The nephritis caused by antikidney serum in rabbits was studied in 30 animals used in a previous investigation of this disease.[18g] Eleven of the rabbits died and 19 were killed at various intervals ranging from 1 to 149 days following the intravenous injection of single or divided dosages of a total of 1.8 to 5.7 ml. per kg. of antikidney serum prepared in ducks. Hematoxylin and

1935-1936. (*d*) Arnott, W. M.; Keller, R. J., and Matthew, G. D.: Experimental Glomerulo-Nephritis Produced by Use of Specific Sera, Edinburgh M. J. **43**:233, 1936. (*e*) Tsuji, S.: Ein Beitrag zur Frage der immun-cytotoxischen Glomerulonephritis. Beitr. path. Anat. **98**:425, 1936-1937. (*f*) Ehrich, W. E.; Wolf, R. E., and Bartol, R. E.: Acute Experimental Glomerular Nephritis in Rabbits: Correlation of Morphological and Functional Changes, J. Exper. Med. **67**:769, 1938. (*g*) Sarre, H.: Pathogenese der menschlichen diffusen Glomerulonephritis, Deutsche med. Wchnschr. **65**:1661, 1939. (*h*) Kay, C. F.: Mechanism by Which Experimental Nephritis is Produced in Rabbits Injected with Nephrotoxic Duck Serum, J. Exper. Med. **72**:559, 1940.

19. Kay, C. F.: Mechanism of Form of Glomerulonephritis: Nephrotoxic Nephritis in Rabbits, Am. J. M. Sc. **204**:483, 1942.

20. Kay, C. F.: Blood Pressure in Experimental Nephritis Produced by Injection of Nephrotoxic Serum, Arch. Path. **29**:57, 1940.

21. Footnote 18 *e, f, h.*

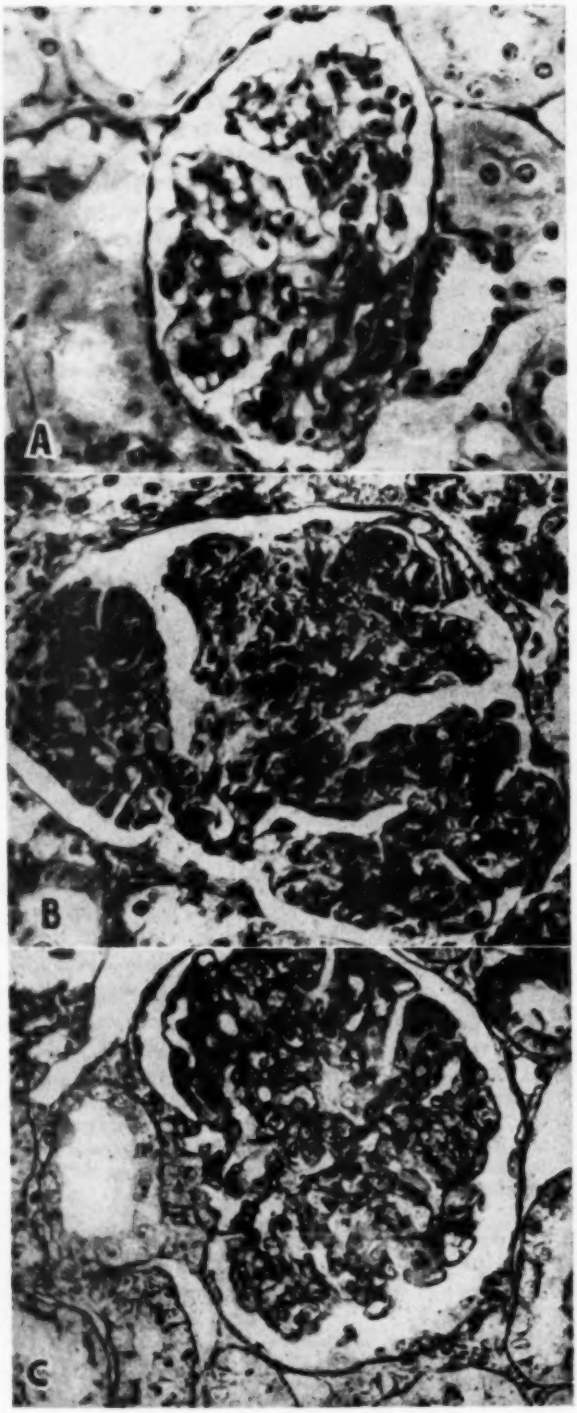


**Figure 3**

*(See legend on opposite page)*

eosin stained sections of the kidneys were examined before and after decolorization and subsequent staining by the methods listed above (p. 464).

*Results.*—Of six rabbits dead or killed during the first week of the experiment, two showed swelling of epithelial cells and slight thickening of the capillary basement membrane. Three of four rabbits dead or killed 8 to 10 days following the injection of serum contained thrombotic material within the capillaries. This stained yellowish with BTS and red with ROPS, indicating the presence of fibrin or fibrinoid. In some places it occluded the capillary lumen; in others it formed a film over the endothelium.

Of eight animals dead or killed 8 to 12 days following injection, all showed varying degrees of endothelial proliferation within the tufts (intracapillary glomerular nephritis) (Fig. 3 *B*). About half of these animals contained exudate in the capsular space of 1 to 53% of their glomeruli (Fig. 4 *A*). The exudate stained mostly yellow with Beyer's trichrome and red with periodic acid-Schiff, indicating the presence of fibrin or fibrinoid, but it contained components staining blue with both stains (hyaluronic acid?). It appeared to be located chiefly between loops, and between loops and capsule (extracapillary glomerular "nephritis"). It was associated in acute cases, with necrosis of loops (Fig. 4 *A*). The epithelial cells were moderately increased in number and size and in part contained granules staining yellow with BTS and red with ROPS. The visceral basement membrane was finely split or moderately thickened in half of these cases (Fig. 3 *C*); in one rabbit sacrificed on the eighth day, the membrane appeared to be normal in spite of a marked increase in number and size of the endothelial cells in all glomeruli and the presence of exudate in the capsular space in 53% of the glomeruli. Sclerosis of capillary loops was difficult to demonstrate. Prominence of the intercapillary space was observed in only two of the eight rabbits.

Rabbits dead or killed after 25 days or later revealed varying degrees of endothelial proliferation and organization by epithelial cells of extracapillary exudate where this had been present (crescent formation). New formation of tubules within the crescents was also observed. Focal sclerosis of capillary loops was seen in occasional glomeruli, but this appeared to be cellular rather than fibrillar in most instances. The basement membrane of the tufts was markedly thickened in some animals, but not altered in others. The intercapillary space was prominent in occasional cases. Most of the rabbits which were killed one and one-half to three months after the injection of serum showed advanced obliteration of 15 to 92% of their glomeruli, epithelial and mesenchymal cells replacing both the tuft and the capsular space. The replacement was cellular rather than fibrous (Fig. 4 *B*).

The tubules contained hyaline casts in 14 and erythrocytes in 11 of 23 rabbits dead or killed after the eighth day. Fatty degeneration of the capsular lining epithelium was observed in 7 and hyaline droplets in 2 of 22 rabbits tested.

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EXPLANATION OF FIGURE 3

Fig. 3.—*A* (Rabbit 566), normal rabbit glomerulus. ROPS. *B* (Rabbit 119), intracapillary glomerular nephritis with marked endothelial proliferation and prominent basement membrane 8 days after two injections of 3.5 ml. each of nephrotoxic serum. BTS. × 450. *C* (Rabbit 121), intracapillary glomerular nephritis with prominent basement membrane 10 days after two injections of 3.5 ml. each of nephrotoxic serum. ROPS.

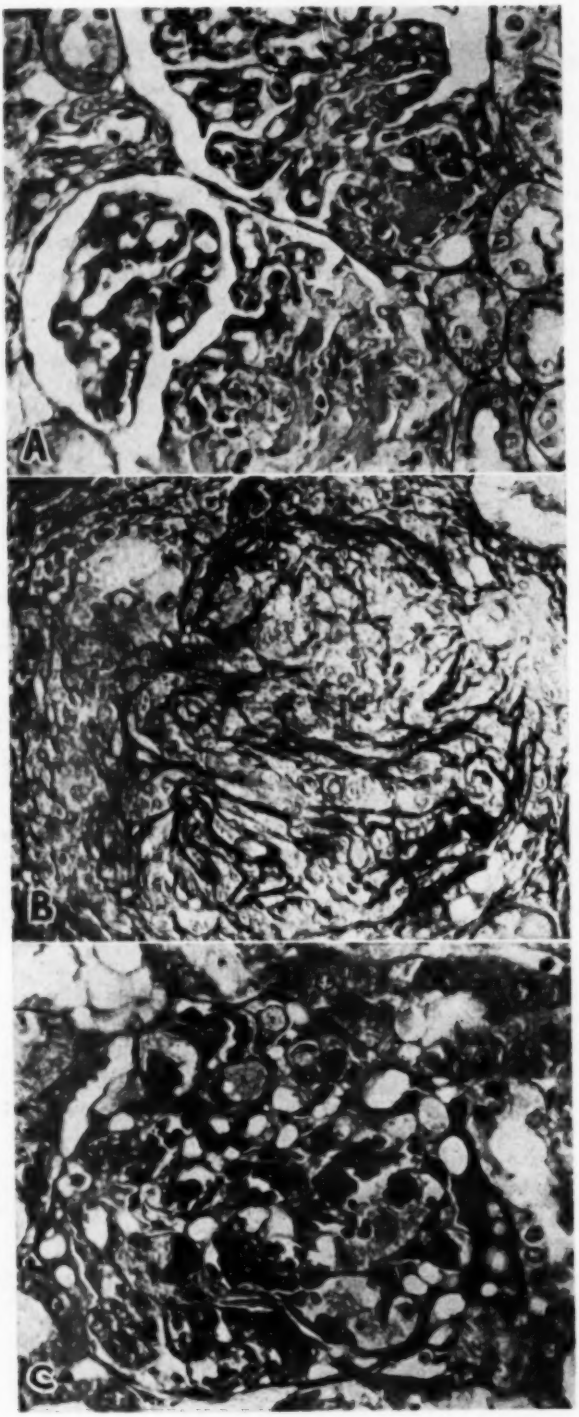


Figure 4

*(See legend on opposite page)*

### FOREIGN PROTEIN NEPHRITIS IN RABBITS

#### PREVIOUS OBSERVATIONS

The renal disease caused by foreign protein in rabbits is likewise believed to resemble diffuse glomerular nephritis in man.[22] Hawn and Janeway[22b] observed this nephritis as early as the seventh day following a single injection of protein. In our animals[22c] swelling of loops and pyknosis of endothelial cells was seen 3 to 5 days after a single injection of horse serum, but cellular proliferation in the tuft and exudation into the capsular space were observed only on the eleventh day. It is true that two of four rabbits studied on the third and fifth day showed increased cellularity of their tufts, but one of these rabbits had pyelonephritis and in the other the cells were too pyknotic for proper analysis. It thus appears that this nephritis, like antikidney serum nephritis, develops after a latent period of approximately one week. This observation and the fact that it is usually associated with periarteritis, carditis, and other manifestations of serum sickness suggest that it is the result of an antigen-antibody reaction in the sense of an Arthus phenomenon.

The clinical manifestations of foreign protein nephritis have hardly been studied. Our rabbits[23] showed a mild transient hyperproteinuria during the first few days following the injection of serum. Later no protein was found in half of a group of rabbits tested 11 days after a single injection of serum. The other half was tested on the 15th day after the injection. Several had proteinuria. Sixty to 120 mg. of protein per 100 ml. of urine was found in two of four rabbits receiving horse serum only, and in one of three desoxycorticosterone-acetate-treated animals. These three rabbits all had severe extracapillary glomerular "nephritis."[24] A rise in blood urea

22. (*a*) Masugi, M., and Sato, Y.: Über die allergische Gewebereaktion der Niere, zugleich ein experimenteller Beitrag zur Pathogenese der diffusen Glomerulonephritis und der Periarteriitis nodosa, Arch. path. Anat. **293**:615, 1934. (*b*) Hawn, C. V. Z., and Janeway, C. A.: Histological and Serological Sequences in Experimental Hypersensitivity, J. Exper. Med. **85**: 571, 1947. (*c*) Ehrich, W. E.; Seifter, J., and Forman, C.: Experimental Serum Disease: Pathogenetic Study, ibid. **89**:23, 1949. (*d*) More, R. H., and Waugh, D.: Diffuse Glomerulonephritis Produced in Rabbits by Massive Injections of Bovine Serum Gamma Globulin, ibid. **89**:541, 1949. (*e*) Wissler, R. W.; Smull, K., and Lesh, J. B.: Effects of Various Horse Serum Fractions in Producing Cardiovascular and Renal Lesions in Rabbits, ibid. **90**:577, 1949. (*f*) Rich, R. A.; Berthong, M., and Bennett, I. L.: Effect of Cortisone upon Experimental Cardiovascular and Renal Lesions Produced by Anaphylactic Hypersensitivity, Bull. Johns Hopkins Hosp. **87**:549, 1950.

23. Forman, C.; Mertens, E.; Graub, M., and Ehrich, W. E.: Blood Histamine, Leukocytes, and Platelets in Experimental Serum Disease in Rabbits, Proc. Soc. Exper. Biol. & Med. **72**:439, 1949.

24. Seifter, J.; Ehrich, W. E.; Begany, A. J., and Warren, G. H.: Effects of Cortisone, Hyaluronidase, Desoxycorticosterone, and Artisone on Experimental Serum Disease in Rabbits, Proc. Soc. Exper. Biol. & Med. **75**:337, 1950.

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#### EXPLANATION OF FIGURE 4

Fig. 4.—*A* (Rabbit 121), recent deposition in the capsular space, and partial organization by epithelial cells of an exudate staining red with periodic acid 10 days after two injections of 3.5 ml. each of nephrotoxic serum. Note necrosis of some of the loops. ROPS. (This is the same field of the same section, decolorized and restained, as that of Fig. 2*d* of a previous publication.[18f]) *B* (Rabbit 36), advanced sclerosis of glomerulus 47 days after one injection of 3.5 ml. of nephrotoxin serum. BTS. *C* (Rabbit 575), partial necrosis of glomerular tuft 15 days after one injection of 15 ml. of horse serum. This animal was treated with cortisone. BTS. × 450.

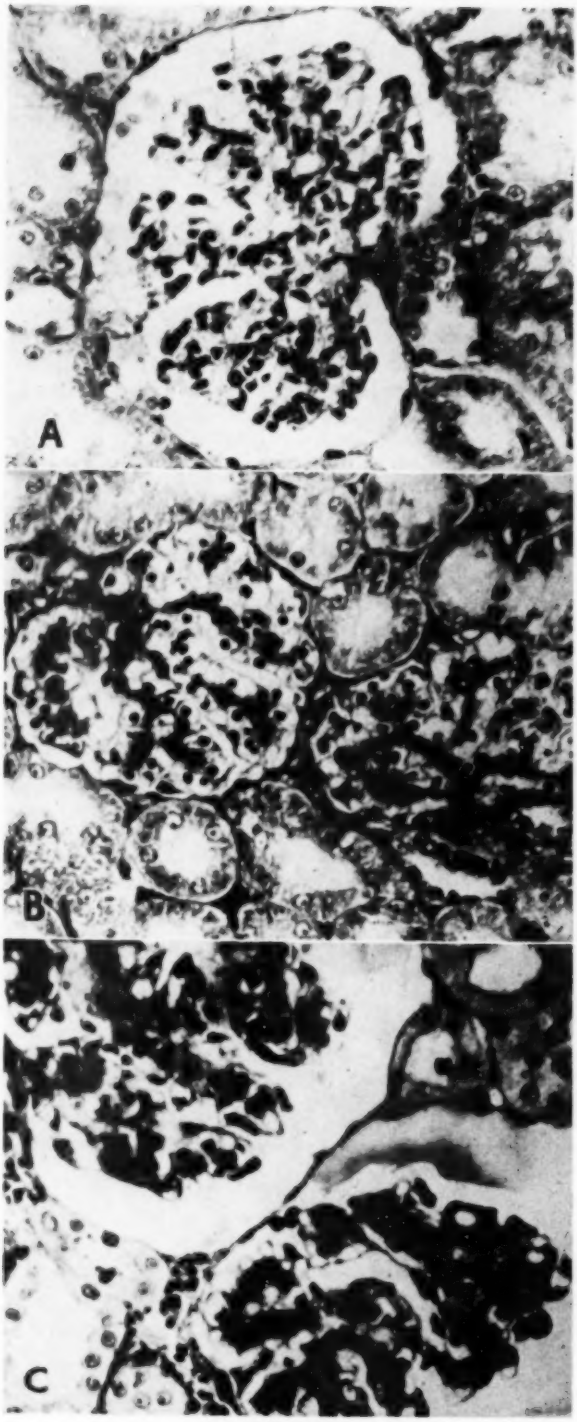


Figure 5

*(See legend on opposite page)*

nitrogen to 187 mg.% was observed in 1 of 18 rabbits studied by Moore and Waugh.[22d]

Microscopically the nephritis has generally been described to be characterized by endothelial proliferation in the tuft and exudation and crescent formation in the capsular space. Some intracapillary coagulation was noted by Masugi and Sato,[22a] Hawn and Janeway,[22b] and More and Waugh.[22d] Thickening and shredding of the basement membrane were described by More and Waugh [22d] and Wissler, Smull and Lesh.[22e] However, the various illustrations which have been published show largely the extracapillary lesion; the changes in the tufts shown in Figure 11 of our previous publication,[22c] and especially in Figure 6 of More and Waugh's paper,[22d] which were interpreted as endothelial proliferation, resemble the alterations to be described and illustrated in the present paper.

While the proliferation within the tufts was diffuse, affecting all glomeruli, and occurred after the first injection of serum, exudation and subsequent crescent formation in the capsular space was focal. In our first series of chinchilla rabbits this was seen only after a second injection.[22c] In subsequent studies of the effect of salicylates upon serum disease in chinchilla rabbits, extracapillary lesions developed in 1 of 15 animals killed 11 days after the first injection of horse serum.[25] A similar observation was made in 3 of 14 albino rabbits killed 15 days after the first injection of large doses of serum only.[24] The distinct character of the two reactions has been confirmed by Wissler, Smull, and Lesh.[22e] It appeared that the extracapillary lesion is due to necrosis of capillary loops, perhaps caused by a severe antigen-antibody reaction.[22c]

### Present Observations

*Materials and Methods.*—The nephritis caused by foreign protein was investigated in 24 rabbits, most of which were previously used in our studies of serum sickness.[24] All animals received one or two injections of 15 to 20 ml. per kilogram of horse serum intravenously. Nine rabbits were treated with 4 mg. of cortisone acetate, and five with 2.5 mg. of desoxycorticosterone acetate twice daily by intramuscular injection beginning one day prior to the injection of serum. The animals were killed 3, 5, 14, and 15 days after a single injection and 5 days after a second injection given 19 days after the first. Sections were cut from blocks of renal tissue embedded in paraffin. All were stained by the methods listed above (p. 464).

*Results.*—Most of the animals killed 14 to 15 days after the first injection of serum showed prominent glomerular changes. The alteration of the tuft is illustrated in Figure 5 *B* and *C*. It can be seen that it is essentially a reaction of the intercapillary space. There is an increase in size and number of the axial mesenchymal cells and an increased deposition of intercellular material staining red

25. Forman, C.; Seifter, J., and Ehrich, W. E.: Effects of Salicylates and Other Drugs on Experimental Serum Disease, J. Allergy **20**:273, 1949.

### Explanation of Figure 5

Fig. 5.—*A* (Rabbit 566), normal rabbit glomerulus. BTS. *B* (Rabbit 266), intercapillary glomerular nephritis with patent capillaries 14 days after one injection of 15 ml. of horse serum. BTS. *C* (Rabbit 579), marked intercapillary glomerular nephritis with mostly patent capillaries 15 days after one injection of 15 ml. of horse serum. This animal was treated with desoxycorticosterone acetate. ROPS. × 450.

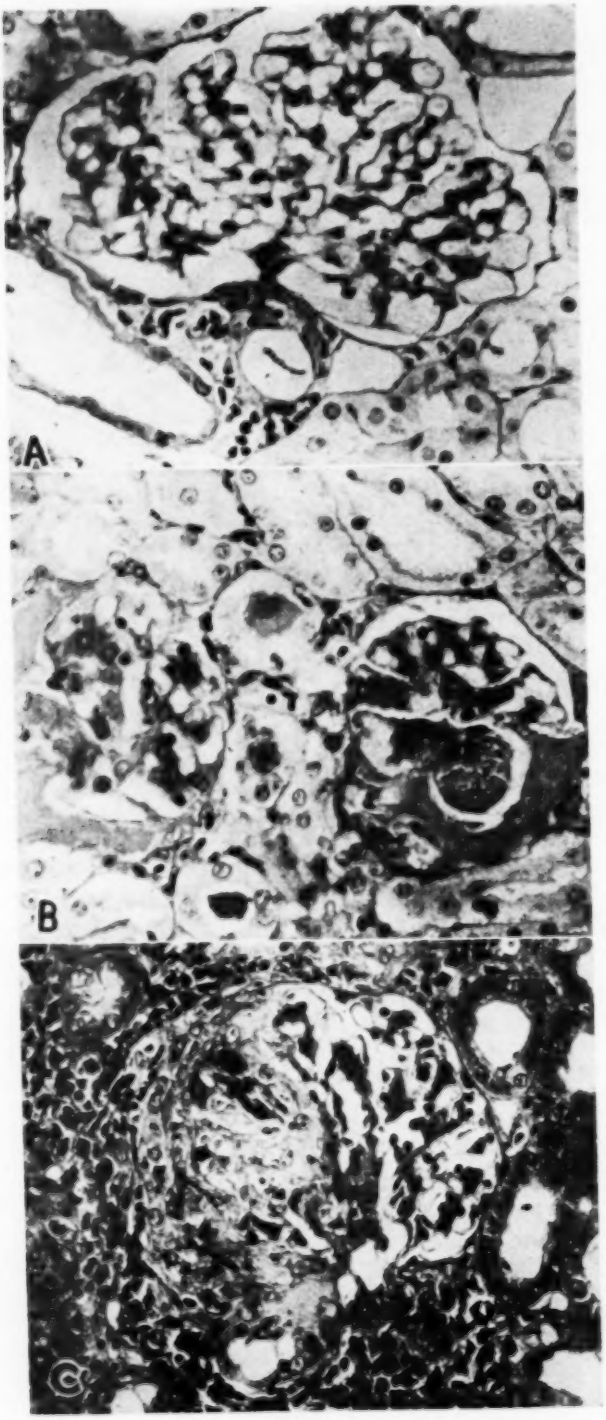


Figure 6

*(See legend on opposite page)*

with ROPS. Various degrees of this reaction were present in all but some cortisone-treated rabbits (Fig. 6*A*); it was most marked in the desoxycorticosterone-acetate-injected animals (Fig. 5*C*). The axis reaction was associated with diminished blood content in the capillaries, although these appeared to be patent. In the cortisone-treated animals, on the other hand, the capillaries were distended with blood (Fig. 6*A*) and occasional glomeruli were partly necrotic (Fig. 4*C*). There was no endothelial proliferation in any of the rabbits. The epithelium tended to be swollen but was free of hyaline granules. The basement membrane of the capillaries was not significantly altered.

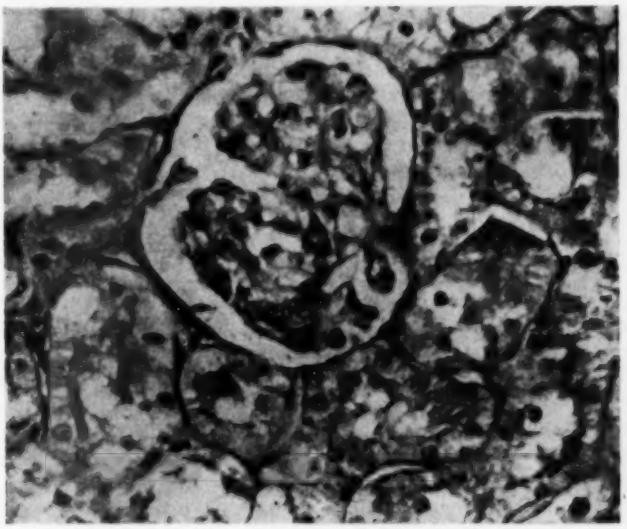

Fig. 7 (Rat VI E 1).—Normal rat glomerulus. ROPS. × 500.

Exudation into the capsular space was observed in two of the four untreated, one of the two desoxycorticosterone-acetate-treated, and most of the cortisone-treated rabbits studied 14 and 15 days after the first injection of serum (Fig. 6*B*). The degree of exudation varied from slight to massive; the extent, from an occasional glomerulus to almost all glomeruli. In some glomeruli the exudate was located chiefly in the space between capsule and tuft; in others within the tuft, apparently between and within the loops, and in still others, in both places. Most of the

EXPLANATION OF FIGURE 6

Fig. 6.—*A* (Rabbit 629), marked hyperemia of glomerular tuft and absence of intercapillary glomerular nephritis 24 days after the first of two injections of 15 ml. each of horse serum. This animal was treated with cortisone. ROPS. *B* (Rabbit 520), recent deposition in the capsular space of an exudate staining mostly red with periodic acid, but partly blue, 17 days after one injection of 15 ml. of horse serum. Note necrosis of some of the loops. This animal was treated with cortisone. ROPS. *C* (Rabbit 624), large crescent and distinct intercapillary reaction 24 days after the first of two injections of 15 ml. each of horse serum. BTS. × 450.

(See legend on opposite page)



exudate stained pink with ROPS and yellow with BTS indicating the presence of fibrin or fibrinoid, but some components were blue with both stains (hyaluronic acid ?). In some animals it contained abundant fibrin fibrils staining black with MMFS. There was no correlation with the axial reaction. In the untreated and the desoxycorticosterone-acetate-treated groups proliferation outweighed exudation; in the cortisone-treated animals exudation was the more prominent change. There was considerable proliferation within the exudate of epithelial cells both of the tufts and of the capsule, especially in the desoxycorticosterone-acetate-treated rabbits, but there was little proliferation of these cells in the animals treated with cortisone.

In the rabbits killed five days after the second injection of serum a similar dualism was observed. Of the two untreated animals analyzed, one showed marked axis reaction and the other widespread exudation, but the exudate now was largely replaced by epithelium (crescent formation) (Fig. 6*C*). There was little fresh exudate and no endothelial proliferation, and the parietal basement membrane was fragmented, thickened, and duplicated.

The tubules were little altered in these animals. Hyaline droplets occurred chiefly in the rabbits with severe extracapillary changes. Hyaline casts were found in most animals. Fat stains were not available in this group.

### ANTIKIDNEY SERUM DISEASE IN RATS

#### Previous Observations

The renal disease caused by antikidney serum in rats was first interpreted as glomerular nephritis. Masugi,[26] using a range of doses, observed three degrees of renal injury depending on the potency and dosage of the serum used. In the mildest cases, four hours after injection he observed in the glomeruli infiltration with polymorphonuclear leucocytes, swelling of endothelium, and masses of protein and occasional fibrin thrombi in the lumen of the capillaries. After 24 hours, the leucocytes were reduced in number, but the endothelial swelling had increased, accompanied by thickening of the capillary membrane and slight swelling of the epithelium. With larger doses or more potent serum 5 to 24 hours after injection the glomerular changes were dominated by the presence of fibrin thrombi or masses of a hyaline protein within the capillaries, which at first covered the inner surface of the capillary wall and later sometimes obliterated the lumen. After 9 to 15 days an increase in the number of endothelial cells appeared within the tufts and in two rats fibrinoid masses and epithelial cells were present in the capsular space. In the severest cases, stasis of blood in the capillaries and pyknosis of the cells of the glomerular tufts were observed. Masugi concluded that this renal disease was the same as acute glomerular nephritis in man.

Smadel,[27] repeating Masugi's experiment, observed that "good" sera caused marked proteinuria, casts, edema, and azotemia but no significant hematuria. Sections of the kidneys revealed extensive tubular degeneration and glomerular changes characterized by thickening of the capillary wall due to swelling of the basement membrane and mild degeneration of endothelium and epithelium. Infiltrative and proliferative changes were practically absent. Other sera caused extensive fibrin

26. Masugi, M.: Über das Wesen der spezifischen Veränderungen der Niere und der Leber durch das Nephrotoxin bzw. das Hepatotoxin, Beitr. path. Anat. **91**:82, 1933.

27. Smadel, J. E.: Experimental Nephritis in Rats Induced by Injections of Anti-Kidney Serum: I. Preparation and Immunological Studies of Nephrotoxin, J. Exper. Med. **64**:921, 1936.

thrombosis of the glomerular capillaries. The urine was filled with blood and protein. If these animals survived for a week, hematuria disappeared, proteinuria continued, casts and edema appeared, and the blood urea nitrogen rose to high levels. Smadel concluded that toxin alone did not cause hematuria or fibrin thrombosis, but these two reactions were due to the presence of different components contained in the sera. In another paper, Smadel [28] reported endothelial proliferation in occasional rats but only after injection of massive doses of serum. He felt that the enlargement of the glomerular tufts was due to swelling of the intercapillary substance instead of to edema of the capillary wall as Masugi had observed. Occasional crescents were noted 30 to 40 days after injection.

In still another publication, Smadel and Farr [29] observed no rise in blood urea nitrogen nor change in urea clearance in rats which received just enough serum to develop marked proteinuria, cylindruria, and edema. The proteinuria came on in a few hours and was maintained for some time at about 40 mg. per milliliter of urine. Casts appeared at the third day, usually in large numbers. Edema was apparent on the fifth day and remained for four to six days; lipemia was usually present. The plasma total proteins usually showed early decrease from 6.5 to 4.5 gm. per 100 ml. Hypertension was observed, but it occurred late. Smadel, like Masugi, spoke of glomerular nephritis.

Solomon, Gardella, Fanger, Dethier, and Ferrebee [30] also noted the absence of hematuria in this experimental disease and found consistent histological changes only in rats which showed heavy proteinuria for at least two weeks, and slight, transitory edema. The changes consisted of widening of the basement membrane by "edema and tissue proliferation." Less frequently they observed mild infiltration with polymorphonuclear leucocytes and exudation and early crescent formation in the capsular spaces. Rats in which moderate to heavy proteinuria developed showed no renal lesions. On the other hand, Hackel, Portfolio, and Kinney [31] reported that they observed a moderate degree of acute glomerular nephritis with increased cellularity, "avascularity," and swelling of the basement membrane four days after the first two divided injections of 10 ml. per kilogram of antikidney serum in rats weighing 250 gm., this in the absence of edema. Similarly, Lippman, Marti, and Campbell [32] observed "mild proliferative glomerular nephritis" with increased cellularity of the tufts beginning as early as 30 minutes following the injection of antikidney antibody in rats weighing 150 gm. Proteinuria increased rapidly during the first 24 hours, reached its peak at 1 week, and then declined. Serum protein, creatinine concentration, and endogenous creatinine clearance calculated in terms of body weight were not significantly changed. There was no edema or ascites.

28. Smadel, J. E.: Experimental Nephritis in Rats Induced by Injection of Anti-Kidney Serum: III. Pathological Studies of the Acute and Chronic Disease, J. Exper. Med. **65**:541, 1937.

29. Smadel, J. E., and Farr, L. E.: Experimental Nephritis in Rats Induced by Injection of Anti-Kidney Serum: II. Clinical and Functional Studies, J. Exper. Med. **65**:527, 1937.

30. Solomon, D. H.; Gardella, J. W.; Fanger, H.; Dethier, F. M., and Ferrebee, J. W.: Nephrotoxic Nephritis in Rats: Evidence for Glomerular Origin of the Kidney Antigen, J. Exper. Med. **90**:267, 1949.

31. Hackel, D. B.; Portfolio, A. G., and Kinney, T. D.: Experimental Nephrotoxic Nephritis in Rat Treated with ACTH or Cortisone, Proc. Soc. Exper. Biol. & Med. **74**:458, 1950.

32. Lippman, R. W.; Marti, H. U., and Campbell, D. H.: Nephrotoxic Globulin Nephritis, A. M. A. Arch. Path. **53**:1, 1952.

Heymann and Lund,[32a] in contrast to all previous observers, pointed out that the disease produced in rats resembles the nephrotic syndrome of childhood more closely than it does glomerular nephritis. More recently [32b] these authors have described in detail the clinical findings in a number of young rats. Interesting highlights were (*a*) development of marked proteinuria, followed by cylindruria, usually within three days after the first injection of serum; (*b*) appearance of edema beginning three to four days after injection and persisting for one to three weeks, and (*c*) development of chronic disease in 19% of the animals with hypertension in 26% of this group. Nonprotein nitrogen rose to 268 mg. per 100 ml., blood urea nitrogen to 330 mg., creatinine to 3.4 mg., total lipids to 19.5 gm., cholesterol to 1.96 gm., and lecithin to 0.725 gm.

Diffuse glomerular lesions were found by Heymann and Lund in 72% of the fulminating and severe cases and in only 11% of the moderately severe cases. The lesions consisted of thickening of the basement membrane, often with a sudanophilic coagulum in the capillaries. Proliferation of endothelium or epithelium was rarely seen during the first week but occurred occasionally in the later part of the first month. Heymann and Lund stated that antikidney serum in rats caused primarily a degenerative disease. If the rats did not recover, the degenerative phase was followed by varying degrees of "fibrous proliferation" in the glomeruli. The degenerative phase was characterized clinically by the nephrotic syndrome; the proliferative phase, by hypertension and uremia. It was concluded that the nephrotic syndrome as observed in infants and children in both its so-called pure and mixed forms is produced experimentally in rats by one and the same procedure.

## Present Observations

*Methods and Materials.*—Antikidney serum disease in rats was produced by the method described originally by Masugi [26] and modified by Heymann and co-workers.[33] Long-Evans rats were anesthetized lightly with an intraperitoneal injection of pentobarbital sodium (nembutal®). As aseptically as possible, the abdominal cavity was opened and the kidneys perfused by allowing warm saline to flow through a needle inserted into the abdominal aorta at the iliac bifurcation. After the perfusion was started, the aorta was occluded cephalad to the renal arteries and the inferior vena cava nicked posterior to the renal veins. This facilitated the process and allowed adequate perfusion. Kidneys so obtained were weighed, mixed with an equal amount of saline and aqueous penicillin solution, 1,000 units per milliliter of extract, and mixed in a Waring blendor® for five minutes. This emulsion was incubated for 24 hours at 36 C. and then stored in 10 ml. vials at 0 C. for indefinite periods.

Four New Zealand rabbits weighing 2,200 gm. were given intraperitoneal injections of 10 ml. of 10, 15, and 20% solutions of the emulsions respectively three times for one week. The next two weeks each animal received three injections of 20% solution. Seven days later the animals were bled by cardiac puncture. The serum was separated by centrifugation and stored in 10 ml. vials at 0 C. after primary detoxification for one-half hour at 56 C.

One hundred twenty-five Long-Evans rats weighing about 50 gm., and five weighing 150 gm. received antikidney serum. For the most part the animals were

33. Heymann, W.; Gilkey, C., and Salehar, M.: Antigenic Property of Renal Cortex, Proc. Soc. Exper. Biol. & Med. **73**:385, 1950. Heymann and Lund.[32a]

studied in groups of 12. Four to six rats in each group received only anti-rat-kidney serum. The remaining rats were given anti-rat-kidney serum of similar type and in similar doses and in addition either 6 mg. of corticotropin per 100 gm. of weight or 2.5 or 12.5 mg. of cortisone per 100 gm. of rat weight daily in two doses or were adrenalectomized one to three days prior to injection. Groups I and II received 1 ml. of anti-rat-kidney serum on two successive days, and Group V received 0.5 ml. in one injection. Groups I, VII, and VIII received serum from different rabbits, and the remaining groups II through XIV received serum obtained from one rabbit after five immunization procedures. The serum from each bleeding was kept in separate vials. All rats received dog pellets and water ad libitum. The adrenalectomized animals were fed the same diet with 5% glucose in saline for drinking water. Urine was obtained by placing the animals in individual metabolism cages overnight without food.

All chemical determinations were performed on single specimens of serum obtained from the abdominal aorta at the time of killing. Total nitrogen was determined by the Kjeldahl method or refractometric determination; blood urea nitrogen, by a method described by Archibald,[34] and albumin-globulin ratio by Howe fractionation; cholesterol was determined directly with acetic anhydride, a modification of the method of Drekter.[35] Quantitative analysis of urinary protein was done by the biuret method.

*Results.*—The clinical and morphological changes in the experimental rats were found to vary with the potency and dosage of the antikidney serum used. Three degrees of severity were distinguished: Group A, the severest type, with death occurring usually in the first week, and with massive edema present by the third day and persisting during the second week in the five surviving animals; Group B, the moderate type, with survival in all except one animal, and with transitory edema (3+) present on the third day but none during the second week of the disease; Group C, the mild group, with survival and no edema. The average weight increase of these rats during the first three days after injection of the serum amounted to 50% in Group A, to almost 40% in Group B, and to less than 10% in Group C. The normal weight increase of the controls was less than 20%. Protein excretion was heavy after three days (2.5 to 30.6 mg. per day), and not so heavy after seven days (0.1 to 24.0 mg. per day), in the first two groups; of the rats of group C all but one showed mild proteinuria, the level ranging from 0.2 to 1.1 mg. per day.

Determinations made on serum collected at the time of killing showed various degrees of hypoproteinemia, hypercholesteremia, and azotemia. Total serum protein ranged from 2.9 to 3.7% in Group A; from 3.4 to 6.9% in Group B, and from 4.4 to 5.9% in Group C. The normal concentration averaged slightly more than 5%. Cholesterol ranged from 195 to 675 mg. per 100 ml. in Group A; from 65 to 328 mg. in Group B, and from 55 to 185 mg. in Group C. The normal concentration averaged slightly less than 100 mg. per 100 ml. Blood urea nitrogen ranged from 57.6 to 93.8 mg. per 100 ml. in Group A; it ranged from 21.5 to 53.1 mg. during the second week of the experiment in Group B, and it was normal (average of 23.5 mg. per 100 ml.) in Group C.

34. Archibald, R. M.: Colorimetric Determination of Urea, J. Biol. Chem. **157**:507, 1945.

35. Drekter, J. J.: New Method for Direct Determination of Cholesterol, Bull. New York M. Coll. **6**:138, 1942.

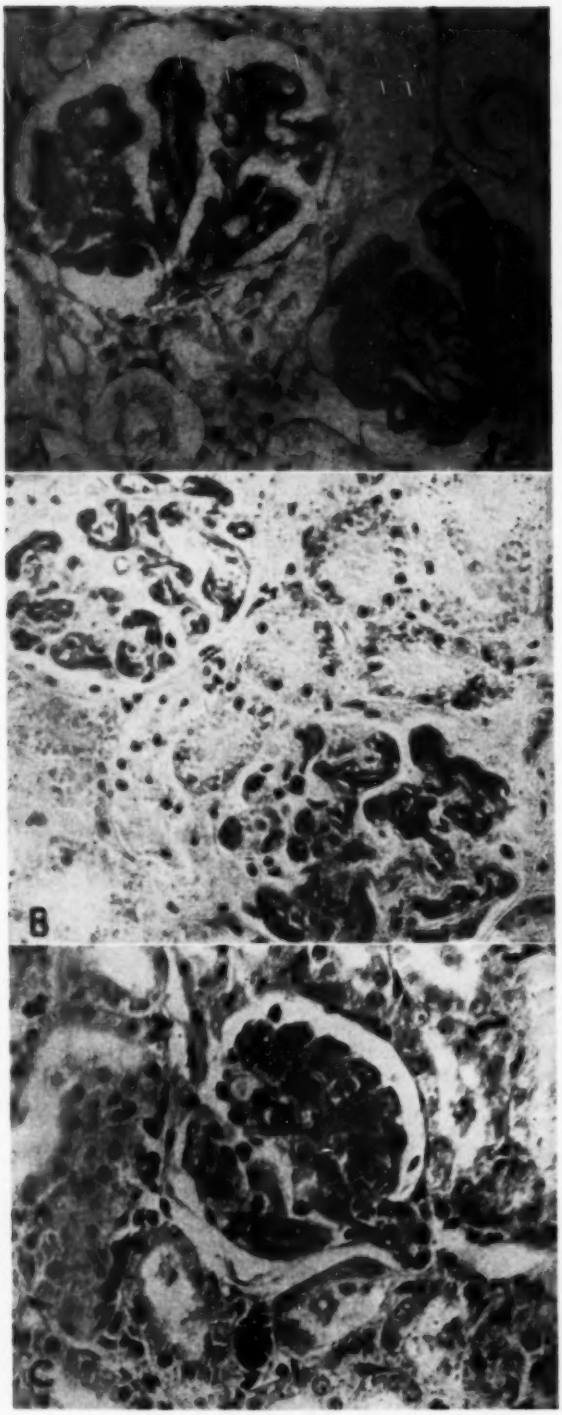


Figure 8

*(See legend on opposite page)*

Morphologically, the three groups also showed significant differences. In Group A the relative kidney weight (organ weight: body weight) increased from a normal average of 11.0 to 14.2 mg. per gram during the first week of the experiment. During the second week the weight dropped to 12.3 mg. per gram. The two rats of the B and C groups killed during the first week had kidney weights of 12.0 and 12.5; those killed during the second week had averages of 12.9 and 12.6.

The relative weight of the adrenal glands also reflected the degree of severity of the disease. In Group A, this weight rose from the control average of 0.24 mg./gm. to 0.60 during the first three days, decreased to 0.31 between the fourth and seventh days and thereafter returned to normal. In Groups B and C, the weight was essentially normal in the few rats tested during the first week and in the second week averaged 0.31 and 0.30 in the two groups respectively.

Microscopic study of the thymus showed the changes expected on the basis of a stress reaction. In Group A, necrosis of all cortical lymphoid cells was seen throughout the 15 days of observation. In Group B, lymphocytolysis was complete during the first week, but dropped to normal during the second. In Group C, the thymus was normal throughout. This behavior was apparent also in the relative weight changes of this organ.

The microscopic changes of the glomeruli and tubules varied with the severity and duration of the disease. In all rats of Group A which survived the first day various degrees of thrombosis developed in the glomerular capillaries. The lumen of the affected vessels was either completely filled by, or its inner walls covered with, a film of a homogeneous protein coagulum (Fig. 8 *A*), at first staining yellow with BTS owing to the presence of abundant fibrin fibrils (Fig. 8 *B*). However, as early as the second day the yellow color began to disappear and was replaced by a blue color (collagenization) (Fig. 8 *C*). After five days neither yellow nor fibrin-positive material could be demonstrated. This thrombotic process was associated with, or followed by, marked thickening of the capillary wall and its basement membrane due to infiltration with the material contained in the capillary lumen (Figs. 9 *A* and *B*). In some places the basement membrane was distinct within the imbibed material; in other places the membrane appeared dissolved therein. Splitting was present but not conspicuous. Thickening of the basement membrane was fully developed one to one and one-half days following injection of the serum. Associated with these changes was a remarkable fatty infiltration of the tufts, both of the cells and of the extracellular components (Fig. 9 *C*). There was no infiltration with polymorphonuclear leucocytes after the first 24 hours and no proliferation of endothelium or epithelium. Many endothelial cells were enlarged and loaded with fat. These changes were still prominent during the second week. Two rats killed at this

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EXPLANATION OF FIGURE 8

Fig. 8.—*A* (Rat IV A 1), lipid nephrosis showing marked thickening of basement membrane one and one-half days after one injection of 1 ml. of antikidney serum in an adrenalectomized rat. The basement membrane is imbibed with periodic acid-positive material. ROPS. *B* (Rat X A 1), lipid nephrosis showing fibrin in glomeruli five days after one injection of 0.1 ml. of antikidney serum. MMFS. *C* (Rat IX C 4), lipid nephrosis showing complete collagenization of deposit in basement membrane five days after one injection of 0.25 ml. of antikidney serum. The material still is periodic acid-positive, but now is negative for fibrin, and stains blue instead of orange with BTS. × 450.

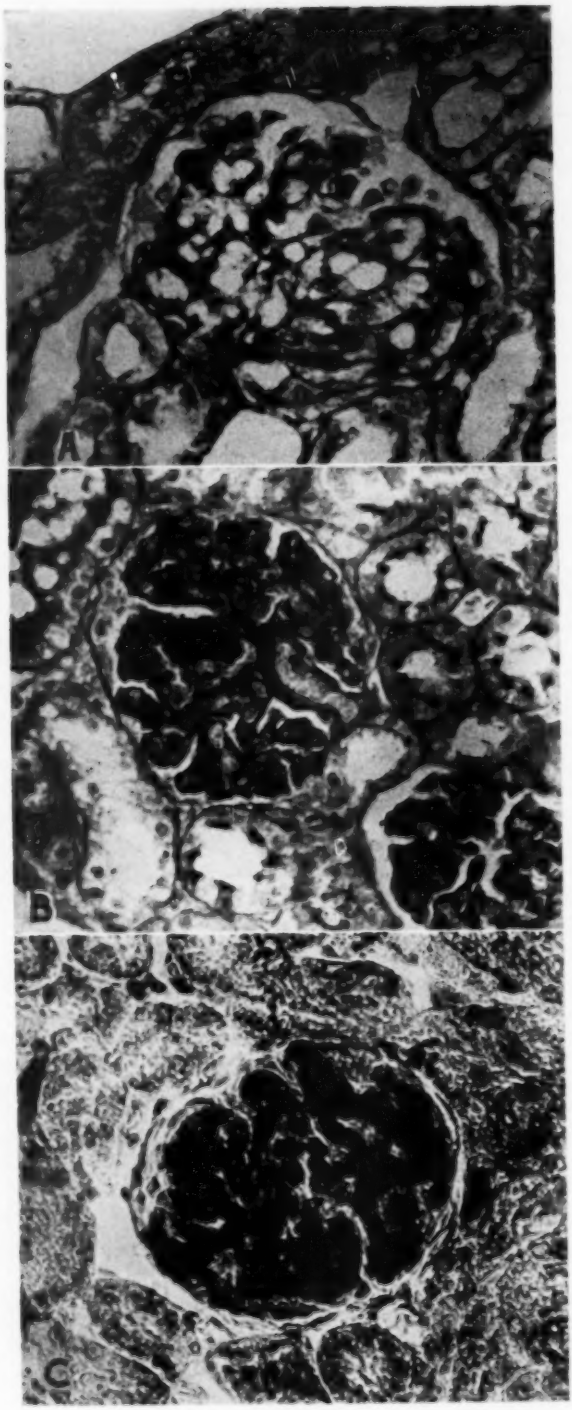


Figure 9

*(See legend on opposite page)*

time showed focal thickening of the basement membrane and mild exudation into the capsular space of a protein resembling that seen in the capillary lumen but with no definite endothelial proliferation.

Some of the tubules were distended and showed focal necrosis as early as the first day following injection; signs of regeneration followed. During the first three days little fat was observed in the epithelium of the proximal convolutions, but after four days considerable amounts were seen. The lumen of the tubules contained hyaline casts which, like the fatty degeneration, were abundant on the fourth day.

In the two animals of Group B examined during the first week, glomerular thrombosis, thickening of the basement membrane, and fatty infiltration were prominent, but not so severe as in Group A; during the second week these changes were still present but greatly diminished. During the second week the prominent features in almost all animals were marked swelling and proliferation of endothelial cells (Fig. 10*A*), and exudation into the capsular space with subsequent epithelial proliferation (Fig. 10*B*). The endothelial proliferation was diffuse, affecting all glomeruli; the exudation into the capsular space and the subsequent crescent formation were focal. Focal sclerosis of tufts was observed in some rats. But they were few, and in most cases it was cellular rather than fibrillar. There was no hemorrhage into the capsular space. The tubules in about half of the animals showed some fatty infiltration and few hyaline casts.

In the one rat of Group C killed during the first week, thrombosis and fatty infiltration of the glomeruli were absent. During the second week, the remaining animals showed a mild degree of endothelial proliferation associated with mild reaction of the basement membrane. Slight exudation into the capsular space was observed in only one rat. Sclerosis of loops was not seen. Fatty infiltration of tubular epithelium occurred in six of nine animals. Casts were not observed.

Adrenalectomy prior to the injection of antikidney serum caused greater susceptibility of the rats to the experimental procedure. Gross edema made its appearance during the first two and one-half days. The weight increase on the third day was less than in the controls. These differences were probably results of the severer stress reaction in these adrenalectomized rats.

Proteinuria was very low in the few animals tested. Similarly, cast formation was greatly depressed. Capillary thrombosis was somewhat diminished, and thickening of the basement membrane and fat content of glomeruli and tubules were markedly decreased in Group A (lipid necrosis), whereas in the other groups (glomerular nephritis) they were elevated rather than depressed. Intracapillary glomerular nephritis was not appreciably altered, but exudation into the capsular space was absent in the six adrenalectomized rats studied in this respect. The

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EXPLANATION OF FIGURE 9

Fig. 9.—*A* (Rat V B 1), lipid nephrosis showing marked thickening of basement membrane and increase in visceral epithelium four days after two injections of 0.5 ml. each of antikidney serum and treated with corticotropin. The diffuse imbibition which was present earlier (Fig. 8*A*) has been replaced by focal thickening. Note similarity to the human lipid nephrosis illustrated in Fig. 2*A*. ROPS. *B* (Rat VIII C 2), lipid nephrosis showing thickening of basement membrane eight days after one injection of 0.5 ml. of antikidney serum. ROPS. *C* (Rat IX C 2), lipid nephrosis showing fat in glomerulus five days after one injection of 0.5 ml. of antikidney serum. Sudan. × 450.

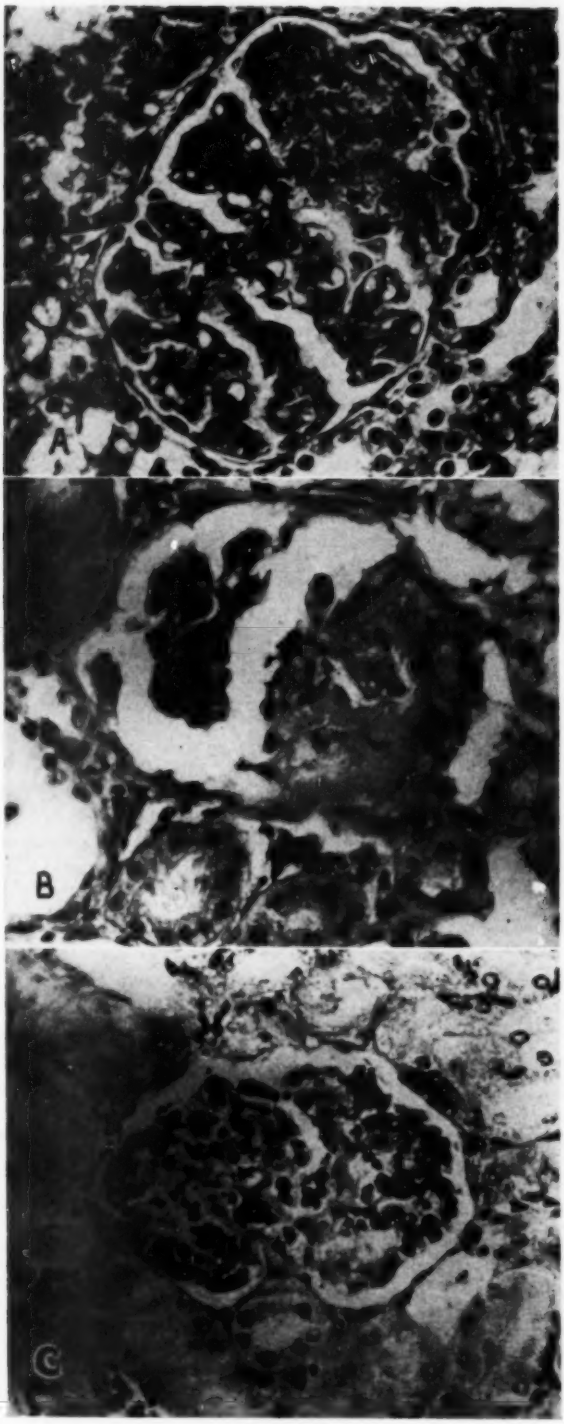


Figure 10

*(See legend on opposite page)*

weight of the kidneys did not differ from that of the serum controls. The weight of the thymus was not depressed, and lymphocytolysis was mild, as was to be expected from the known effect of adrenalectomy upon lymphoid tissue.

Daily injections of 2.5 mg. of cortisone per 100 gm. of body weight caused less edema grossly and as measured by weight increase on the third day. Total serum protein tended to be higher during the second week of the experiment, and proteinuria and cast formation were depressed in most animals studied. Serum cholesterol was somewhat higher in most of the eight animals tested, but fatty infiltration of the glomeruli was markedly depressed. Capillary thrombosis, thickening of the basement membrane, intracapillary glomerular nephritis, and exudation into the capsular space were all markedly depressed. The weight of the kidneys was increased rather than decreased during the second week of the experiment, while the weight of the adrenals was lower and atrophy of the thymus was severer than in the serum controls. The latter is in keeping with the known effects of cortisone.

Daily injections of 12.5 mg. per 100 gm. of body weight of cortisone caused an increase in capillary thrombosis and thickening of the basement membrane. Also increased were fatty infiltration of glomeruli and tubules and cast formation during the first week of the experiment. This dose, however, caused marked renal disturbances also in untreated control rats. Morphologically there was marked hyperemia of glomeruli, and clinically, proteinuria. The weight of the kidneys was considerably larger than that of the serum controls, while the weight of the adrenals was smaller and atrophy of the thymus was severer. The latter was to be expected from the known action of this hormone.

Daily injections of 6 mg. per 100 gm. body weight of corticotropin, like 2.5 mg. of cortisone, depressed edema grossly and as measured by weight increase on the third day. Total serum protein tended to be lower during the second week. Cast formation was severer during the first week. This is in keeping with our observation of hyperemia of the glomeruli and proteinuria in normal control rats receiving this dose of corticotropin. Capillary thrombosis, thickening of the basement membrane, and fatty infiltration of glomeruli and tubules were not appreciably different from those serum controls. The kidney weight was greater and the adrenal weight much greater than in the serum controls. The latter is in keeping with the known effect of corticotropin upon these organs.

## INTERPRETATION

*Diffuse Glomerular Nephritis.*—Our observations in acute and subacute glomerular nephritis in man and anti-kidney-serum nephritis in rabbits are in accord with the view that these diseases are the same, both functionally and morphologically. The majority of patients recover promptly, but in some renal insufficiency develops and they die.

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EXPLANATION OF FIGURE 10

Fig. 10.—*A* (Rat X C 2), intracapillary glomerular nephritis 13 days after one injection of 0.1 ml. of antikidney serum to adrenalectomized rat. Hematoxylin and eosin. *B* (Rat II B 6), extracapillary glomerular "nephritis" 15 days after two injections of 1.0 ml. each of antikidney serum. ROPS. *C* (Rat VI C 2), marked hyperemia of glomerulus following injection of seven mg. of cortisone per kilogram of body weight to control rat. BTS. × 45.

The glomerular changes begin with diffuse infiltration of the tufts by polymorphonuclear leucocytes; this is associated with, or followed by, diffuse endothelial proliferation (intracapillary glomerular nephritis). Thrombosis and subsequent sclerosis of loops and exudation and subsequent crescent formation in the capsular space, in contrast to leucocytic infiltration and endothelial proliferation, may be present or absent. If present, they may affect a few, many, or almost all glomeruli. Hence they are focal phenomena.

While the nephritis caused by antikidney serum in rabbits appeared to be the same as glomerular nephritis in man, the nephritis caused by foreign protein differed from this disease both clinically and morphologically. Significant degrees of hyperproteinuria and a rise in blood urea nitrogen were found only in occasional animals. There was no endothelial proliferation, but the glomerular alteration consisted of a diffuse reaction of the mesenchymal cells in the intercapillary space (intercapillary glomerular nephritis). As in intracapillary nephritis, exudation and subsequent crescent formation in the capsular space were observed only in a certain number of these animals. The extent of this complication varied from an occasional glomerulus to almost all glomeruli.

Our observation that nephritis caused by foreign protein is basically an intercapillary glomerular nephritis is in keeping with the fact that it is part of serum sickness, a systemic disease of the mesenchyme connecting organized structures (collagen disease).[36] The only connective tissue contained in the glomerulus is located in the intercapillary space. Our observation of prominence of this space in human patients with rheumatic fever suggests that intercapillary glomerular nephritis occurs in man as well.

*Human Lipid Nephrosis.*—The first four of our eight patients with the nephrotic syndrome, who died 18 days to 6 months after onset of the disease, fulfill all the criteria of genuine lipid nephrosis. None had hematuria. Two had no rise in blood urea nitrogen. One had no elevation of blood pressure. It is true that blood urea nitrogen was elevated in two cases and blood pressure in three, but these rises were mild and transient, or terminal. This finding is at variance with Volhard's criteria [8] but in keeping with recent observations.[37]

Morphologically, the four patients all showed alteration of the basement membrane of the glomerular tuft as observed by Bell [17] and others.[38] There were focal thickening and splitting of this membrane, thickening being more prominent than splitting. One patient (Case 3) who never had a rise in blood urea nitrogen or blood pressure showed marked thickening but hardly any splitting. There was no evidence microscopically of glomerular infiltration with polymorphonuclear leucocytes or of endothelial proliferation as seen in cases of hemorrhagic nephritis. If these recognized changes of nephritis were present earlier in the course of the disease, they disappeared completely and rapidly (within 18 days in Case 1).

Our observations on the four patients dying after one and one-half to three and one-half years are in agreement with the statement of Ellis [13] that patients with the nephrotic syndrome who do not die early and do not recover ultimately show a picture indistinguishable from that seen in patients dying of chronic glomerular

36. Ehrich, W. E.: Nature of Collagen Diseases, Am. Heart J. **43**:121, 1952.

37. Barnett.[1] Blackman.[11] Blackman.[12]

38. Allen.[2] McManus.[16e]

nephritis. Unlike the previous four patients, all showed evidence of progressive renal failure clinically and many glomerular scars and fresh and hyalinized crescents morphologically. However, this experience should not be generalized. Some of Blackman's patients [11] who lived up to two years after onset of the disease never showed progressive rise in blood urea nitrogen or blood pressure and at autopsy were free of endothelial proliferation or crescent formation. Similarly a patient with lipid nephrosis of 17 years' duration at no time had renal failure or elevation of blood pressure, and when he died, of peritonitis, was singularly free of intracapillary or extracapillary lesions.[39] Observations like these indicate that lipid nephrosis may continue for many years without showing a picture indistinguishable from that of chronic glomerular nephritis.

Conditions like those in our latter four cases are generally diagnosed as glomerular nephritis with the nephrotic syndrome (subchronic stage [8] or degenerative stage [9b]) or as "mixed" forms ("nephronephritis").[14] This diagnosis is made, clinically, because of progressive renal failure, and morphologically, because of the presence of glomerular scars and of crescents or their hyaline substitutes. However, crescents are observed commonly in focal embolic glomerular nephritis, intercapillary glomerular nephritis, lupus erythematosus disseminatus, and malignant arteriolar sclerosis. Similarly, renal failure may be caused by many different disorders of the kidneys. These changes therefore are not diagnostic of glomerular nephritis.

It is true that the renal disease in Case 6 may have been a "mixed form," that is, glomerular nephritis with the nephrotic syndrome, for it began with a febrile rash, there was early progressive renal failure, and microscopic examination showed endothelial proliferation. But it may well be that at least two of the remaining three patients, namely those in whom progressive renal failure developed very late during the disease (Cases 7 and 8), never had glomerular nephritis, but they suffered from pure lipid nephrosis with associated thrombosis of loops, exudation into the capsular space, and subsequent obliteration of glomeruli. There is no evidence in Ellis' report which would exclude this possibility. There is no sound basis, therefore, for his conclusion that a separation of lipid nephrosis from glomerular nephritis is not justified.

*Antikidney Serum Disease in Rats.*—Our experimental observations in rats are in accord with the conclusion of Heymann and Lund [3] that antikidney serum in this species may cause a renal disease resembling lipid nephrosis rather than glomerular nephritis. However, they differ from the findings of these and other recent observers in two important respects. First, whereas no significant glomerular changes were found by most observers in their animals with mild disease, all our animals showed glomerular lesions when sections were stained with ROPS. Second, it was found that the more severely diseased rats could be separated into two distinct groups: Group A, which showed the nephrotic syndrome throughout the period of observation without revealing glomerular nephritis, and Group B, in which glomerular nephritis rather than lipid nephrosis developed during the second week, after a brief episode of nephrosis during the first.

Our clinical observations on these rats agree best with those of Heymann and Lund [3b] and those obtained by Smadel with "good" sera.[27] Antikidney serum in

39. (*a*) McKay, E. M., and Johnston, C.: Lipoid Nephrosis: Report of Case of Unusual Duration, Arch. Int. Med. **45**:734, 1930. (*b*) Ehrich, W. E.: Lipoid Nephrosis of Unusual Duration: Pathologic-Anatomic Report, ibid. **45**:749, 1930.

large doses or if potent enough caused marked proteinuria, hypoproteinemia, hypercholesteremia, and edema commencing during the first three days after injection. In smaller doses or if less potent it produced slight elevation of blood urea nitrogen and other evidence of glomerular nephritis after a latent period of one week. The nitrogen was increased also during the first week in the rats in which the nephrotic syndrome developed. This may have been due to the glomerular thrombosis which was found in these animals, to the demonstrated stress reaction, or to both. Blood pressure was not determined in our rats. Smadel and Farr [29] as well as Heymann and Lund [3b] found hypertension only late in the course of the disease. These observations seem to suggest that hypertension was a feature not of the nephrotic syndrome but rather of progressive renal failure.

Comparison of the various clinical observations on our rats showed good correlation between edema, on the one hand, and proteinuria, hypoproteinemia, and hypercholesteremia, on the other. An increase in body weight of over 40% on the third day was associated in most cases with a urinary protein excretion of 50 mg. per day or more. Gross edema at death was found in all rats showing serum protein levels below 4 gm. per 100 ml. and cholesterol levels above 200 mg. per 100 ml.

Our morphological findings correlate most closely with those of Masugi.[26] In both his and our rats, there was observed an initial infiltration of the tufts with polymorphonuclear leucocytes. In groups A and B this was followed soon after injection of the serum by accumulation of protein, including fibrin, and fat within the capillaries. The latter was associated with, or followed by, swelling of the capillary membrane. In Groups B and C, endothelial swelling and proliferation developed during the second week. As in intracapillary and intercapillary glomerular nephritis, subsequent sclerosis of loops, and exudation and subsequent proliferation of epithelial cells in the capsular space, were observed in varying numbers of glomeruli in a certain number of the rats.

The observation of Smadel [29] that the glomerular enlargement was due to swelling of the intercapillary substance rather than to "edema" of the capillary wall has not been confirmed by these studies. It is true that in some rats receiving large doses of antikidney serum the intercapillary space became prominent. But this was observed only during the second week of the experiment; it was interpreted as a foreign protein reaction analogous to the nephritis produced in rabbits by the injection of horse serum.

Of the morphological alterations, glomerular thrombosis, thickening of the basement membrane, and fat content were well correlated in the animals observed in the first week. There was also an interesting correlation between the fat content of the glomeruli and of the tubules and between tubular fat and cast formation during the last half of the first week. Intracapillary and extracapillary glomerular lesions occurred simultaneously during the second week of the experiment.

Comparison of the clinical and morphological data showed that during the first week of the disease the degree of edema, proteinuria, and hypercholesteremia corresponded to the degree of thickening of the basement membrane. During the second week proteinuria varied with the degree of intracapillary glomerular nephritis. The cholesterol level in the blood compared moderately with the amount of glomerular fat content, but no correlation existed between cholesterol levels and tubular fat.

These various observations seem to show that thrombosis, thickening of the basement membrane, and fatty infiltration of the glomeruli are closely related phenomena. Fatty infiltration of the tubules and cast formation reached their peaks somewhat later, suggesting that they were sequels of the glomerular alteration. The observation of close correlation between the nephrotic syndrome, clinically, and thickening of the basement membrane, morphologically, indicates a pathogenetic relationship between the two.

*Comparison of Antikidney Serum Disease in Rats with Human Lipid Nephrosis and Glomerular Nephritis.*—Antikidney serum disease in rats and lipid nephrosis and glomerular nephritis in man seem to have no significant differences clinically. It is true that our rat nephrosis was an acute and serious disease which often caused azotemia and death in a few days; but in previous experiments, Smadel and Farr [29] were able to produce a less acute and more prolonged nephrosis without rise in blood urea nitrogen or change in urea clearance.

Morphologically, both the nephrotic and the nephritic changes observed in the rats were essentially the same as in man. Both diseases were associated with changes in the basement membrane. In lipid nephrosis, the change consisted chiefly of an irregular thickening of the membrane. In glomerular nephritis it consisted of splitting rather than thickening.

The close correlation of the nephrotic syndrome with alteration of the basement membrane in both man and rats indicates that lipid nephrosis resembles the nephrotic syndrome in chronic glomerular nephritis, amyloidosis, intercapillary glomerular sclerosis, and lupus erythematosus disseminatus in that it too is associated with a characteristic glomerular lesion. The fact that in lipid nephrosis in rats this alteration preceded the peak of proteinuria and other clinical changes indicates that the glomerular alteration in this case is the cause and not a sequel of the proteinuria, as was believed by Fahr.[15]

It has been demonstrated in rats that lipid nephrosis and glomerular nephritis can be dissociated. The rat nephrosis was not associated with glomerular infiltration by polymorphonuclear leucocytes or with endothelial proliferation. Similarly, our first four human patients with the nephrotic syndrome showed no clinical or morphological evidence of nephritis. These observations suggest that in man, as in rats, lipid nephrosis may occur as an entity from the beginning.

*The Basement Membrane in Glomerular Nephritis and Lipid Nephrosis.*—Bell's [17] splitting of the basement membrane, and McManus' [16c] new formation by endothelial cells of reticular fibers in the capillary wall, in acute and subacute glomerular nephritis, on the one hand, and Bell's focal and diffuse thickening, McManus' localized thickening, and Allen's [2] soft swelling of this membrane, in cases with the nephrotic syndrome, on the other hand, were confirmed by this study. This difference in the reaction of the basement membrane may be explained by differences in the pathogenesis of the two diseases. While the thickening in lipid nephrosis is obviously due to imbibition with plasma components of the capillary wall, the splitting can probably be explained by the activity of the proliferating endothelial cells or other connective tissue elements.

In rats with the nephrotic syndrome dead or killed during the first few days of the experiment, the thickened basement membrane stained red with ROPS and yellowish to orange with BTS. It also contained fine fibers staining black with

MMFS. It thus behaved like fibrinoid. During the second to the fifth day the yellowish to orange color of BTS changed to blue while the black color of MMFS disappeared. This change is interpreted as an expression of collagenization. The rapid occurrence of this change explains our failure to observe fibrinoid in human cases of lipid nephrosis.

*Sclerosis of Loops in Glomerular Nephritis and Lipid Nephrosis.*—Sclerosis of loops occurred in two forms: (*a*) as convolutions of shrivelled thickened basement membrane, and (*b*) as a dense network of mostly fine fibers staining like collagen and containing nuclei in their meshes. The former tended to affect all loops of a given glomerulus (diffuse sclerosis). The latter was limited mostly to a few loops of an affected glomerulus (focal sclerosis).

In the causation of diffuse glomerular sclerosis, anoxia may well be a factor, as suggested by McManus.[16c] The focal sclerosis, on the other hand, appears to be due to organization of thrombotic material within capillary loops. Thrombosis has often been described in acute glomerular nephritis in man [15]; its occurrence in human lipid nephrosis was also noted.[11] Focal sclerosis of loops is commonly seen in human glomerular nephritis, and, as shown in this paper, also in lipid nephrosis. Thrombosis of loops and subsequent focal sclerosis was a common feature in our rabbits and rats with glomerular nephritis and lipid nephrosis. In experimental lipid nephrosis in rats, the fibrin contained in the capillaries was transformed within a few days into an amorphous material. If this was not washed away, it was replaced by fibrils staining black with GST. The first silver fibrils were observed two days after injection of serum.

The belief of Smadel [27] that thrombosis in rats is caused by a component in the antikidney serum different from "nephrotoxin" is not supported by convincing evidence. There is no record in this paper of microscopic studies of rats killed or dead two to three days after injection of his "good" sera. The later absence of fibrin is explained by the demonstration in this paper that it is rapidly converted into collagen and thereafter escapes recognition.

*Exudation into the Capsular Space and Crescent Formation.*—Formation of crescents was observed in our cases only in glomeruli containing coagulated glucoprotein in their capsular space. Hence, they appear to be the result of organization of exudate by visceral and parietal epithelial cells. The fate of crescents is hyalinosis. The statement of Bell [17] that they are replaced by collagen fibers derived from epithelial cells could not be confirmed. Instead, we found replacement by a layered but amorphous material staining blue instead of red with ROPS.

Crescent formation occurred in the rabbit, as in man, during the acute phase of glomerular nephritis; it developed simultaneously with endothelial proliferation. The widely accepted view that it is a phenomenon of the subacute stage must therefore be abandoned.

The presence of crescents is interpreted usually as evidence of extracapillary glomerular "nephritis." This interpretation may be acceptable if they occur together with inflammation of the glomerular tuft. However, it is well known that crescents occur in many glomerular diseases. They are frequently found in focal embolic glomerular nephritis and in intercapillary glomerular nephritis caused by foreign protein. They are seen also in purely degenerative diseases, such as lupus erythematosus disseminatus and malignant nephrosclerosis. Hence they appear to be

unspecific complications of alterations of the tufts; they are not necessarily inflammatory," but they are reparatory and hence should not be looked upon as evidence of nephritis.

Exudation into the capsular space occurred also in three of the six rats in our Group A which were killed or died during the second week of acute lipid nephrosis. "Fibrin clots" in the capsular space were observed in children with lipid nephrosis who died from causes other than renal failure.[11] Crescents were found in our four children with lipid nephrosis in whom progressive renal failure developed and death occurred 18 months to 3½ years after onset of the disease (Cases 5 to 8). These observations indicate that exudation into the capsular space and crescent formation occur in lipid nephrosis as well as in glomerular nephritis and other renal diseases.

Exudation into the capsular space and subsequent crescent formation occur in the same diseases in which thrombosis or embolism, and subsequent sclerosis of glomerular loops, are commonly seen. Our patient who died during the subacute stage of glomerular nephritis showed not only the most marked crescent formation of all our human patients but also the most extensive thrombosis within the capillaries. On the other hand, our patients with acute glomerular nephritis revealed neither crescents nor thrombosis. These observations indicate that the extracapillary lesion is related to intracapillary thrombosis. Figures 4 *A*, 6 *B*, 6 *C*, and 10 *B* suggest that necrosis of thrombosed loops may be a factor in its causation.

*Effects of Adrenalectomy and of Corticotropin and Adrenal Cortex Hormones upon Experimental Renal Disease.*—The observed enhancement by desoxycorticosterone acetate and depression by cortisone of both the intercapillary reaction and the epithelial proliferation in nephritis due to foreign protein in rabbits is in accord with the known effect of these hormones upon tissue growth.[40] Our observation of distention of capillary loops and partial necrosis of tufts by cortisone is in keeping with similar observations by Rich, Berthrong, and Bennett.[23f]

In rats, potent doses of antikidney serum (Group A) caused a marked increase in adrenal weight during the first three days and marked lymphocytolysis and reduction in thymus weight thereafter, indicating a marked stress reaction. Doses which produced mild glomerular nephritis but no nephrosis (Group C) caused no significant stress reaction.

Adrenalectomy prevented lymphocytolysis and reduction in thymus weight. Lipid nephrosis appeared to be less severe. In none of the adrenalectomized rats did exudation into the capsular space develop, whereas in these rats endothelial proliferation was the same as, if not severer than, in the controls. This observation supports the view that glomerular nephritis and lipid nephrosis are different diseases and that the extracapillary lesion is independent of intracapillary proliferation.

The administration of 2.5 mg. of cortisone per 100 gm. of body weight caused less proteinuria and edema, and a marked depression of capillary thrombosis, thickening of basement membrane, and fat content of the glomeruli in lipid nephrosis, and apparently also caused less of the intracapillary and extracapillary changes in glomerular nephritis. On the other hand, 12.5 gm. of cortisone aggravated rather than

40. (*a*) Plotz, C. M.; Howes, E. L.; Meyer, K.; Blunt, J. W.; Lattes, R., and Ragen, C.: Effect of Hyperadrenal State on Connective Tissue, Am. J. Path. **26**:709, 1950. (*b*) Spain, D. M.; Molomut, N., and Haber, A.: Effect of Cortisone on Formation of Granulation Tissue in Mice, ibid. **26**:710, 1950.

lessened the rat disease, but this dose caused glomerular hyperemia and proteinuria in normal controls. The administration of 6 mg. of corticotropin had no significant effect.

The depressing action of moderate doses of cortisone upon lipid nephrosis and glomerular nephritis in rats is of interest in connection with the demonstrated increase in plasma volume and glomerular filtration rate following administration of corticotropin to human patients with these diseases.[41] Our observation that large doses aggravate rather than depress the rat disease is in agreement with the finding by Hackel and co-workers [31] of increased proteinuria in antikidney serum nephritis in rats treated with large doses of this hormone.

*Clinical Considerations.*—The mechanisms which "cause certain kinds of glomerular nephritis to become relentlessly progressive while others are completely resolved" [2] are now apparent. While the intracapillary proliferation of endothelium may subside without severe consequences, thrombosis and subsequent sclerosis of loops lead to partial or complete obliteration of the glomerulus. Similarly, extracapillary exudation and subsequent crescent formation may be followed by attempts at regeneration, such as the formation of abortive tubules within the crescents, as emphasized by McManus.[16c] However, restitution does not occur; instead it is followed by destruction of the glomerulus. This course of events explains why crescents are prominent in patients who die weeks or months after onset of the disease (subacute stage of Volhard). Those patients in whom endothelial proliferation only (intracapillary glomerular nephritis) develops usually recover. Those whose glomeruli are injured severely enough for many scars or a significant number of crescents or both to develop die sooner or later.

It is true that obstruction of glomeruli may occur also as the result of extensive endothelial proliferation in acute cases. The prominence of glomerular scars and of crescents or their hyaline substitutes during subacute or chronic stages, however, suggests that it is usually the presence or absence of this complication that determines the fate of a patient with glomerular nephritis.

The same complication also explains the "paradoxical" situation [9] that patients with clinically "severer" acute glomerular nephritis with gross hematuria usually recover, whereas in those with "milder" disease without hematuria the condition progresses to subacute and chronic stages. Hematuria can occur only in the presence of open capillaries and capsular spaces. If either is occluded by thrombi or scars, or by exudate or crescents, diapedesis of erythrocytes and their descent through the tubules cannot take place.

The fate of patients with lipid nephrosis appears to be similarly determined. If the injury of the basement membrane is associated with extensive thrombosis and subsequent sclerosis of loops, or with extensive extracapillary exudation and crescent formation, or both, the patient is bound to die with progressive renal failure. If this is insignificant or absent, recovery may ensue. Cases of the latter kind have been followed for almost 20 years.[42]

41. Barnett, H. L.; Forman, C. W.; McNamara, H.; McCrory, W. W.; Rapoport, M.; Michie, A. J., and Barbero, G.: Effect of Adrenocorticotrophic Hormone on Children with Nephrotic Syndrome: II. Physiologic Observations on Discrete Kidney Functions and Plasma Volume, J. Clin. Invest. **30**:227, 1951.

42. McKay, Johnson, and Ehrich.[10] Addis.[9b]

This interpretation furnishes a new explanation for the observation of Ellis [13] that patients with lipid nephrosis who do not die early during the disease and who do not recover ultimately show a picture indistinguishable from glomerular nephritis. They may well have had lipid nephrosis with considerable capillary thrombosis and exudation into the capsular space followed by crescent formation and subsequent obliteration of glomeruli.

*Pathogenetic Considerations.*—The studies reported here strongly support the view of Volhard [5] and Fahr [15] and of Addis and Oliver [9] that diffuse hemorrhagic glomerular nephritis is a disease entity. It has been shown that the rabbit antikidney serum nephritis resembles the acute, subacute, and chronic stages of Volhard and Fahr, the initial and terminal stages of Addis and Oliver, the Type I nephritis of Ellis,[13] and the diffuse proliferative nephritis of Bell.[17] It does not resemble the subchronic stage of Volhard and Fahr, the degenerative stage of Addis and Oliver, the Type II nephritis of Ellis, or the membranous glomerular nephritis of Bell.

It has been further demonstrated that the glomerular nephritis caused by foreign protein does not resemble hemorrhagic nephritis but is another entity; it is characterized by reaction of the intercapillary space. Evidence has been presented to show that diffuse intercapillary glomerular nephritis occurs in man as well as in animals.

Studies of human lipid nephrosis failed to reveal evidence of preceding glomerular nephritis. Similarly, experimental lipid nephrosis in rats (Group A) was singularly free of inflammatory changes during the period of observation. Instead, both the human and the experimental disease were characterized by fibrinoid degeneration of the capillary wall, an alteration which is generally regarded as a degenerative disturbance. These observations strongly suggest that Volhard and Fahr and Addis and Oliver were right when they considered lipid nephrosis "a single distinct disease entity which is not preceded by unrecognized acute glomerular nephritis." [1]

Several writers [42] now speak of "mixed" forms or "nephronephritis." Previous writers spoke of a subchronic stage,[44] a degenerative stage [9] or nephritis Type II.[13] It is true that many of these patients in reality have lipid nephrosis complicated by sclerosis of loops and crescent formation in the capsular space. However, it is conceivable that in some cases glomerular nephritis may follow lipid nephrosis,[45] or that lipid nephrosis may develop in the wake of glomerular nephritis (subchronic or degenerative stage of glomerular nephritis),[46] or that the two conditions may arise simultaneously. It is of interest that the rats of our Group B in which glomerular nephritis developed during the second week showed an abortive nephrosis during the first week. Similar findings have been reported in man.[6]

The fact that both glomerular nephritis and lipid nephrosis can be produced by the same experimental procedure suggests an etiological relationship.[3b] The specificity of antikidney serum has been well established by various absorption techniques.[47] Recently, the seat of the antigen in the kidney has been more closely

43. Heymann and Lund.[3] Galán.[14a]

44. Volhard.[5] Fahr.[15]

45. Allen.[2] Heymann and Lund.[3b]

46. Volhard.[5] Addis and Oliver.[9] Fahr.[15]

47. (*a*) Pearce, R. M.: Experimental Study of Nephrotoxins, Univ. Pennsylvania M. Bull. **16**:217, 1903-1904. (*b*) Wilson, G. W., and Oliver, J.: Experiments of Production of Specific Antisera for Infections of Unknown Cause: III. Nephrotoxins; Their Specificity as Demonstrated by Method of Selective Absorption, J. Exper. Med. **32**:183, 1920. Smadel.[27] Smadel.[28]

identified. Heymann and co-workers [48] were able to produce the disease in rats more frequently with serum produced with the use of medullary portions. Pressman and co-workers [48] demonstrated the localization of radioiodinated globulin fraction of antirat kidney serum in the glomeruli. Greenspon and Krakower [49] prepared an antiserum against basement membrane material isolated by means of centrifugation technics. The membranous extract was 20 times more antigenic than the cellular part of the glomerulus. Recent studies by Cole and associates [50] suggest that the antigen is possibly a protein in combination with a polysaccharide or lipid. These various observations are in keeping with the view that lipid nephrosis, like glomerular nephritis, is primarily a glomerular disease.

In humans, lipid nephrosis develops under the same conditions as glomerular nephritis.[8] There is good reason to believe that both diseases are caused by hemolytic streptococci. It thus appears that the human diseases are likewise related etiologically.

However, pathogenetically, glomerular nephritis and lipid nephrosis are not the same. Antikidney serum nephritis in rabbits and rats developed only after a latent period of one week, i. e., after the foreign serum injected with the specific antiglomerular antibody had elicited antibody in these animals directed against the serum. On the other hand, lipid nephrosis in rats came on at once, obviously as a result of injury of the basement membrane by the antikidney serum.

It has been suggested that the latent period in antikidney serum nephritis in rabbits is due to species difference either of the host or of the donor. It was shown here that the development of glomerular nephritis in rats also requires a latent period of one week. It was found that rabbits do not tolerate more than 5 ml. per kg. of antikidney serum, whereas rats survive 30 ml. per kg. or more and that in rats which received large doses or more potent sera chiefly the nephrotic syndrome developed, while in rats receiving smaller doses of the same serum chiefly glomerular nephritis developed. These observations suggest that the observed differences in the reaction of rabbits and rats were due to differences in the doses of serum used rather than to species differences.

Morphologically, glomerular nephritis is characterized by infiltration with polymorphonuclear leucocytes and subsequent endothelial proliferation; hence, it is a productive inflammation. On the other hand, lipid nephrosis is characterized by fibrinoid degeneration of the capillary wall, i. e., a regressive alteration.

These various observations suggest that glomerular nephritis and lipid nephrosis, though etiologically related, are otherwise very different diseases. It appears that they are brought about by two different immunological reactions, resembling the Arthus reaction in glomerular nephritis and possibly the Shwartzman reaction in lipid nephrosis. The fact that the lesion in glomerular nephritis is productive while that in lipid nephrosis is regressive suggests that the damage of the glomeruli in lipid nephrosis is either of a different nature or of greater severity than in glomer-

48. Pressman, D.; Hill, R. F., and Foote, F. W.: Zone of Localization of Anti-Mouse Kidney Serum as Determined by Radioautographs, Science **109**:65, 1949.

49. Greenspon, S. A., and Krakower, C. A.: Localization of Antigen Responsible for Production of Nephrotoxin Serum Within Isolated Renal Glomerulus, Federation Proc. **10**: 355, 1951.

50. Cole, L. R.; Cromartie, W. J., and Watson, D. W.: Specific Soluble Substance Involved in Nephrotoxic Nephritis, Proc. Soc. Exper. Biol. & Med. **77**:498, 1951.

ular nephritis. It may well be that lipid nephrosis and glomerular nephritis represent two different reactions to the same noxious material, as suggested by Bell,[17] but it is equally possible that the two are caused by different components contained in this material. Experiments designed to solve this question are under way.

## SUMMARY AND CONCLUSIONS

The experimental diseases caused by antikidney serum and horse serum in rabbits and rats have been compared with human glomerular nephritis and lipid nephrosis.

It was found that the antikidney serum disease in rabbits closely resembles human diffuse hemorrhagic glomerular nephritis both clinically and morphologically. Both the rabbit and the human nephritis are primarily an intracapillary glomerular nephritis characterized by diffuse endothelial proliferation with fine "splitting" of the basement membrane. The nephritis caused by horse serum in rabbits, on the other hand, is an intercapillary glomerular nephritis characterized by a diffuse reaction of the intercapillary space without significant alteration of the basement membrane. The latter disease was observed also in man.

The renal disease caused by antikidney serum in rats varies with the dose and potency of the serum. Large doses or potent sera cause lipid nephrosis commencing immediately after injection. In smaller doses, or if less potent, antikidney serum produces intracapillary glomerular nephritis after a latent period of one week.

The lipid nephrosis in rats, like human lipid nephrosis, is characterized morphologically by fibrinoid degeneration of the glomerular membrane. This regressive alteration may last, in man, for many years without causing renal failure. In rats it is not preceded by or associated with proliferation of endothelium.

Both intracapillary and intercapillary glomerular nephritis and lipid nephrosis either occur in pure form or may be complicated by focal thrombosis and subsequent sclerosis of capillary loops, by focal exudation and subsequent crescent formation in the capsular space, or by both. While patients with the pure form usually recover, those with complications, if these are severe enough, die eventually with renal failure. It thus appears that the fate of patients with diffuse glomerular nephritis and lipid nephrosis depends to a large extent upon the degree of complicating thrombosis of loops and of exudation into the capsular space.

The results reported here support the view of Volhard and Fahr and Addis and Oliver that lipid nephrosis and glomerular nephritis are not the same. The demonstration that the two diseases may be caused by the same experimental procedure suggests that they are related etiologically. However, pathogenetically they are very different diseases; glomerular nephritis is characterized by productive inflammation and lipid nephrosis by fibrinoid degeneration.

Dr. J. McManus assisted in preparing the manuscript. Dr. J. Kidd supplied the kidneys of six patients, and Drs. P. Klemperer and J. Oliver, those of the other two.

# News and Comment

**Course on Legal Medicine.**—A course on legal medicine will be given at the Armed Forces Institute of Pathology, Dec. 1-5, 1952. The course is designed to cover the major problems of medicolegal nature which confront pathologists. Inasmuch as there is considerable overlap of endeavor necessary for the successful completion of a medicolegal case, the data from such varied fields as toxicology and police science will be presented in sufficient detail for orientation of the pathologist in these pursuits. The emphasis of the course, however, will be directed toward the practical management of medicolegal problems by pathologists.

The speakers have been selected because of their vast experience and outstanding work in the field of legal medicine.

The course is open to pathologists of the armed forces and of federal agencies and civilian pathologists. The attendance quota is not yet completely filled. Applications to attend this course in forensic pathology should be forwarded to The Director, Armed Forces Institute of Pathology, Washington 25, D. C.

**American Academy of Forensic Sciences.**—The American Academy of Forensic Sciences announces its Fifth Annual Meeting, which will be held Feb. 26 to 28, 1953 at the Drake Hotel, Chicago. All persons planning to present papers should submit titles to Dr. Milton Helpern, program chairman, 106 E. 85th St., New York 28, before Dec. 1, 1952.

This program will be preceded by a one-day meeting of the Society for the Advancement of Criminology on Feb. 24, 1953, at Northwestern University Law School, Chicago.

**International Society for Geographic Pathology.**—Robert A. Moore, M.D., who is Dean of Washington University School of Medicine, St. Louis, and Edward Mallinckrodt Professor of Pathology, was elected President of the International Society for Geographic Pathology at the last meeting of the society, in Liége, Belgium. The next meeting, concerned principally with cancer, will be held in September, 1954, in Washington, D. C.

**Appointment.**—Arthur T. Hertig, M.D., has been appointed Shattuck Professor of Pathological Anatomy at Harvard Medical School. Dr. Hertig succeeds S. Burt Wolbach, M.D., who retired in 1947 after 25 years of service. Dr. Hertig is pathologist at the Free Hospital for Women and pathologist and senior obstetrician at the Boston Lying-in Hospital.

# Books

A Textbook of Histology. By Harvey Ernest Jordan, A.M., Ph.D., Sc.D., Professor of Anatomy, retired, University of Virginia. Ninth edition. Price, $9.00. Pp. 687, with 609 illustrations. Appleton-Century-Crofts, Inc., New York, 1952.

As the succession of editions indicates, this has been a popular textbook of histology. The preface of its ninth edition points to "extensive revisions, considerable elimination of text and incorporation of important advances during recent years." However, a critical review of the textual contents and number and quality of figures leaves one with considerable disappointment that such claims are hardly justified. A comparison with the eighth revised edition (1947) reveals exactly the same pagination of all nineteen chapters. Textual changes are decidedly minor, and the presentation of facts and newer concepts is often inadequate. Examples of such deficiencies are to be found in the discussion of the nature of the ground substance in areolar connective tissue, in the detailed consideration of the histological features of various joints, and in the discussion of the derivation of the lymphatic system, the origin and role of the dendritic melanoblasts in cutaneous pigmentation, and the newer findings concerning the structural details of the kidney.

Limited additions have been made to the illustrations by including electron photomicrographs of myofibrils. Recent advances in this field might have justified the incorporation of such photographs portraying structural details of other tissues and organs. The book contains few multicolored plates. The mere outlay of a single color over essentially black and white figures cannot be considered as a high-class illustration process. The list of references to the literature has been extended by half a page. In comparison with other modern texts on histology this book appears to have remained stagnant.

Experimentelle Histopathologie: Ein Einführungskurs. By Prof. Dr. H. Meesen, Direktor des pathologischen Instituts der Medizin, Akademie Düsseldorf. Price, 22.50 German marks. Pp. 153, with 125 illustrations. Georg Thieme, Diemershaldenstrasse 47, (14a) Stuttgart-O, Germany; agents for the United States, Grune & Stratton, Inc., 381 4th Ave., New York 16, 1952.

Almost by definition, an experiment in histopathology differs in a radical way from one in physiology or pharmacology by reason of the time factor involved. There are, it is true, pathological events (wounds, emboli, erythemas, shock) that may be demonstrated in a few minutes and some that may be demonstrated in a few hours or days (bacterial invasion, nephrosis, alloxan diabetes, hormone effects). But more readily come to mind changes that demand a certain inexorable passage of time: tumor induction by most methods; changes of radiation, starvation, vitamin deprivation, castration, fat deposition, nerve regeneration, and amyloid production; sclerotic change. Not a few of these are still research problems, the outcome of which is by no means cut and dried, the lesion not bound to appear. This volume presents more than a hundred exercises in experimental pathology. These are drawn from modern sources and the references given. It would certainly be frequently necessary to consult the original. Each lesion is accompanied in most cases by an adequate illustration. Relatively few of these experiments could be called "student exercises" in the usual sense; not only are they time-consuming, but highly specialized techniques are frequently demanded. Rather, these point the way for extended research along the various lines. For a demonstration course in experimental pathology, this represents a comprehensive list from which to choose some problems. But, failing active and cooperative research departments in several disciplines, even a fraction of these experiments would constitute a major undertaking, comprising as they do exercises in radiation, ultrasound, tissue culture, transplantation, cytology, nutrition, and endocrinology, as well as "pathology." At the least, this compilation illustrates in a graphic way the current scope of experimental histopathology.

THE 1951 YEAR BOOK OF PATHOLOGY AND CLINICAL PATHOLOGY (JANUARY-DECEMBER, 1951). Pathology: Edited by Howard T. Karsner, M.D., LL.D., Medical Research Advisor to the Surgeon General, United States Navy. Clinical Pathology: Edited by Arthur Hawley Sanford, M.A., M.D., Emeritus Professor of Clinical Pathology, Mayo Foundation; Emeritus Consultant, Division of Clinical Laboratories, Mayo Clinic; Director of Laboratories, Rochester (Minn.) State Hospital. Price, $5.50. Pp. 454, with 147 illustrations. The Year Book Publishers, Inc., 200 E. Illinois St., Chicago 11, 1952.

The 1951 Year Book of Pathology and Clinical Pathology continues the high standards set by its predecessors. As in recent volumes, there are several special articles: on epidemic hemorrhagic fever, by Steer and Hullinghorst; on the pathogenesis of arterial lesions, by Holman; on atherosclerosis, lipoproteins, and arteriosclerosis, by Gofman and others, and on avian arteriosclerosis by Katz and others. These brief special reviews have considerable interest, and in future volumes perhaps their number can be expanded. Apart from the special articles, the abstracts follow the usual forms, with division by system, and frequent brief pointed editorial comment. The section on clinical pathology excellently covers recent material in the various aspects of this specialty.

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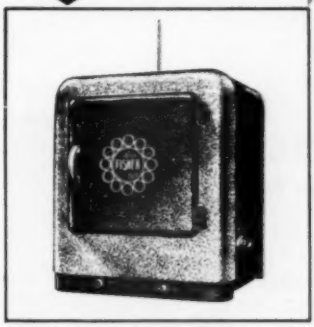

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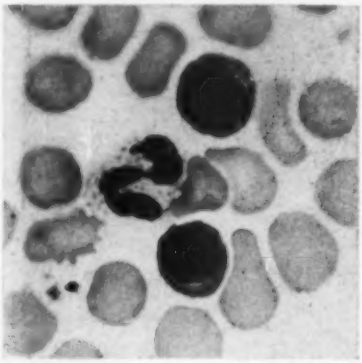

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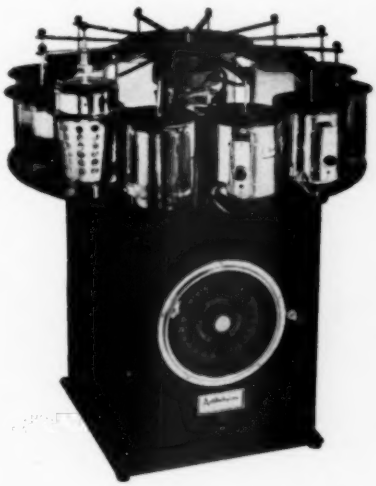

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